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Emerging prospects for the disease-modifying treatment of Alzheimer's disease

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Abstract

The currently approved therapies for Alzheimer's disease (AD) in the US are designed to modify the function of specific neurotransmitter systems in the brain. While these palliative treatments can benefit some patients for a period of time, they do not halt the relentless cognitive and behavioral deterioration that characterize this neurodegenerative disorder. Consequently, much current research on AD is directed toward illuminating the disease process itself, particularly the abnormal accumulation of certain proteins in brain: the amyloid-β protein (Aβ) in senile plaques and cerebral blood vessels, and the tau protein in neurofibrillary tangles. Genetic, biochemical and pathologic evidence now favors a primary role of Aβ aggregation in the Alzheimer proteopathic cascade, and studies in mice indicate that lowering the amount of this protein in brain can be beneficial. Recently, Aβ-immunization therapy has emerged as a particularly promising therapeutic option for treating Alzheimer's disease, but unexpected treatment-related side-effects are an overriding issue. These adverse events were not anticipated from preclinical studies with rodents; hence, more biologically relevant models, such as nonhuman primates, are needed to test the safety and efficacy of novel therapies for Alzheimer's disease.

Keywords: Amyloid; Cerebral amyloid angiopathy; Primate; Proteopathy; Senile plaque; Tau; Transgenic mice; Vaccination

1. Alzheimer's disease: pathology and current treatment options

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the inexorable decline of cognitive function, alterations in judgment, perception and personality, and ultimately the loss of essential qualities that define a human existence. The many signs and symptoms of AD reflect the dysfunction of diverse brain regions. The recognized histopathological hallmarks of AD are numerous senile plaques and neurofibrillary tangles in specific regions of the brain (Fig. 1). *Senile plaques* comprise an extracellular core of aggregated, fibrillar β-amyloid peptide (Aβ) accompanied, to varying degrees, by microglial cells, astrocytes, and dystrophic neuronal processes [1,2]. Aβ is cleaved from the β-amyloid precursor protein (βAPP) by enzymes generically dubbed β-secretase (or β-amyloid cleaving enzyme, BACE) and γ-secretase [2,3]. *Neurofibrillary tangles* (NFT) consist of intracellular fibrils made of aberrantly polymerized, hyperphosphorylated *tau*, a protein that normally participates as a monomer in the assembly and stabilization of microtubules [4]. In addition to plaques and tangles, an elaborate and inconsistent assortment of other lesions are found in the AD brain, including cerebral β-amyloid angiopathy (CAA; Fig. 2A), granulovacuolar degeneration, neuropil threads, Lewy bodies, and selective but widespread degeneration of neurons and their connections [1,2]. Longitudinal magnetic resonance imaging (MRI) studies show a progressive,

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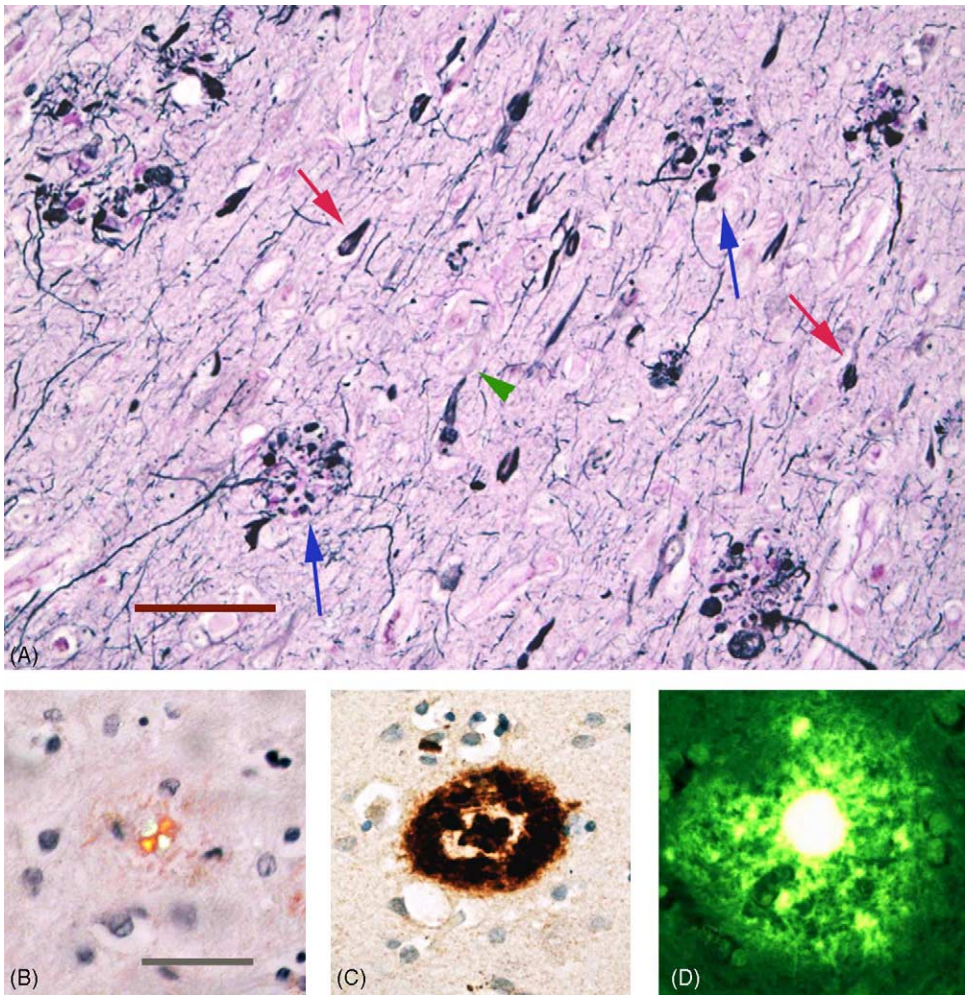

Fig. 1. The canonical pathology of Alzheimer's disease. (A) Senile plaques and neurofibrillary tangles in a hippocampal section stained with the Naoumenko–Feigen silver impregnation method and counterstained with periodic acid-Schiff (PAS). Two classical senile plaques are indicated by blue arrows; they consist of an extracellular core of amyloid (stained pinkish-red by PAS) encircled by black, distended neuronal processes (neurites). Diffuse deposits of Aβ usually lack neurites and are not stained by this method. Note also the intracellular NFT (two are marked by red arrows); a pale, normal-appearing neuron is designated by a green arrowhead. Bar = 100 μm. (B) A neocortical senile plaque stained with Congo Red, the definitive stain for classically defined ''amyloid'' (regardless of the identity of the protein component); the photograph was taken with crossed polarizing filters, which produce a Maltese-cruciform pattern of green-orange birefringence in compact, fibrillar amyloid deposits (the plaque core, center); Nissl counterstain. (C) A cortical senile plaque immunostained with antibody 10D5 to amino acids 3–7 of Aβ. Note the central core of β-amyloid, surrounded by a halo, and then an outer ring of Aβ, which is typical of many dense-cored plaques in AD; Nissl counterstain. (D) A cortical plaque stained with Thioflavin-T, a fluorescent dye that binds to generic amyloid. The core is intensely fluorescent, and the peripheral, more diffuse deposits also bind Thioflavin. Bar in B = 50 μm for B, C and D.

disease-specific loss of brain substance over the course of the disease [5]. Remarkably, AD tends to afflict certain neurons and brain regions preferentially, and to leave others relatively spared.

The complex cerebral degeneration in AD has been fertile ground for the cultivation of hypotheses on the pathogenesis of the disorder. In the 1970s and 80s, methodological advances began to shed light on perturbations of various transmitter systems in the AD brain. Prominent among these was the degeneration of the basal forebrain acetylcholinergic system (nucleus basalis of Meynert and nucleus of the diagonal band of Broca) [6]. This discovery, in the context of experimental evidence for a role of acetylcholine in memory, swiftly led to the development of cholinesterase inhibitors (tacrine, donepezil, rivastigmine and galantamine) for the treatment of AD [2,7,8]. More recently, memantine, a modulator of ionotropic neurotransmitter receptors targeted primarily to the *N*-methyl-D-aspartate receptor, was approved in the U.S. for treatment of moderate-to-severe AD [9]. These agents, which were developed to correct specific transmitter deficiencies, can benefit some AD patients for a limited period of time [9,10]. However, owing to the extensive and multifocal nature of neurodegeneration in AD, the effects of transmitter modulators are modest.

Numerous other interventions have been, or are being, tested as preventives or therapies for AD, including anti-inflammatory drugs, vitamins, hormone replacement ther-

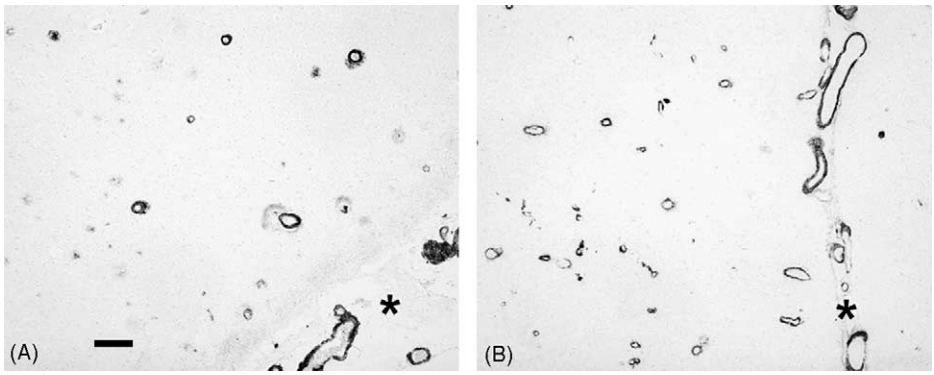

Fig. 2. Neocortical cerebral amyloid angiopathy in a human with AD (A) and in a 23-year old squirrel monkey (B) immunostained with antibodies to Aβ. Note in both instances numerous circular and ovoid vascular profiles that are Aβ-positive in brain parenchyma and in the sulci (✱). Bar = 100 μm.

apy, metal chelating agents, statins, and herbal supplements [2,9]. To date, there is no compelling evidence that any of these treatments will stop the functional deterioration of AD. It is apparent that a successful disease-modifying therapy must address the essential pathogenic process; current hypotheses implicate the abnormal accumulation of specific proteins in the brain. Strategies designed to impede the buildup of Aβ in brain are beginning to show promise in the fight against AD, both in the laboratory and in the clinic.

2. The Aβ cascade: out of order comes chaos

From the labyrinthine pathology of AD has emerged a unifying theme with profound implications for treatment: The ordered aggregation of Aβ plays a seminal role in the Alzheimer proteopathic cascade. Three principal arguments support this view: (1) the magnitude of Aβ deposition in the AD brain implicates this protein in the disease process; (2) the most common autosomal dominant forms of AD all involve mutations in βAPP or in the presenilins (which constitute the probable catalytic subunit of the gamma secretase complex [11]); and (3) all known genetic and environmental risk factors for AD increase the production of Aβ and/or its tendency to aggregate [3,12]. Abnormalities of tau play a crucial role in the clinical manifestations of AD; indeed, the number of NFT correlates strongly with the degree of cognitive impairment [1]. However, genetic and experimental studies furnish persuasive clues that tauopathy occurs secondarily to Aβ multimerization in the pathogenic sequence; specifically, mutations in βAPP or presenilin result in autosomal dominant AD with plaques and tangles, whereas mutations in the gene for tau cause tauopathies with NFT, but generally in the absence of senile plaques [3,12]. This pathogenic unidirectionality is confirmed by transgenic mouse models in which the expression of excess Aβ expedites tauopathy, but not vice versa. Normally, transgenic mouse models of Aβ accumulation do not have AD-like neuronal loss or NFT; however, when Aβ is expressed (or injected) in the brains of mice that express human tau protein, tauopathy is significantly augmented [13].

According to the Aβ cascade hypothesis, the abnormal accrual of multimeric Aβ triggers a succession of events leading to amyloid plaques, NFT, inflammation, loss of neurons and synapses, and possibly the emergence of other lesions as well [3]. These changes can, in turn, feed back into the cascade and further exacerbate the pathogenic process. How Aβ initiates the disease remains controversial, but a crucial occurrence in the Aβ cascade now appears to be a gain of toxic function mediated by misfolded and aggregated Aβ. In this regard, there may be informative parallels in the pathogenesis of AD and seemingly disparate diseases, each involving a unique protein, such as triplet nucleotide repeat disorders, Parkinson's disease, prion diseases, and a host of others [12].

Based largely on in vitro studies of synthetic peptides, the formation of β-amyloid fibrils is thought to entail first a change in the secondary structure of the protein from alpha helix/random coil to a structure high in β-sheet content, causing the molecule to misfold into a highly stable (and noxious) state. This corrupted molecule then propagates its structural features by interacting with, and thereby converting, like molecules, possibly with the assistance of co-factors. The misfolded molecules assemble into small oligomers and protofibrils (also known as Aβ-derived diffusible ligands, or ADDLs [14]) as well as amyloid fibrils, which are the fundamental ultrastructural constituents of compact senile plaque cores and cerebral amyloid angiopathy. Whereas senile plaques and CAA are a conspicuous result of the Aβ-aggregation process, the cellular damage inflicted by Aβ may result largely (but not exclusively) from the intra- and/or extracellular action of small, cryptic, pre-fibrillar assemblies of the molecule [12,15] (below). Regardless of how Aβ aggregation disrupts brain function, the genetic, pathologic and biochemical evidence that it is a primary event in the disease process argues that reducing Aβ aggregation and toxicity is a compelling strategy for disease-modifying treatment of AD.

3. Therapeutic options for Alzheimer's disease proteopathy

There are three general approaches to obstructing the Aβ cascade: (1) inhibit the production of Aβ; (2) block the aggregation and toxicity of Aβ; and (3) stimulate the degradation and removal of Aβ.

3.1. Inhibition of Aβ production

Experimental studies in animal models have shown that secretase inhibition lowers the amount of Aβ in brain, and can thereby reduce the formation of Aβ aggregates [8,16]. Thus, both BACE and gamma secretase are viable therapeutic targets, but they are turning out to be refractory to drug discovery, for different reasons. Potent, small molecule gamma secretase inhibitors have been discovered that diminish the Aβ load in brain; unfortunately, one of several other substrates for gamma secretase is Notch, which, when cleaved by gamma secretase, releases a crucial signaling protein in cellular proliferation and differentiation pathways. Gamma secretase therefore is important for embryogenesis, and gamma secretase knockout mice are not viable. In adults, inhibiting the cleavage of Notch has potentially serious side-effects, such as reduced hematopoiesis. However, the extent to which this issue will be problematic in AD patients remains an open question; postnatal inactivation of neuronal presenilin-1 has only mild phenotypic effects on mice [17], and such conditional knockout animals do not develop Aβ-plaques when crossed with βAPP-transgenic mice [15]. Selective inhibitors of gamma secretase cleavage of βAPP (as opposed to Notch) have been reported. Even so, inhibition of gamma secretase results in the accumulation of C-terminal βAPP-fragments that are potentially neurotoxic. In addition, conditional knockout of both presenilin-1 and presenilin-2 in mouse forebrain results in severe, age-related functional and morphologic brain abnormalities [18]. Thus, the promise of gamma secretase inhibitors for AD therapy is, for the time being, constrained by possible mechanism-related side-effects.

From a safety standpoint, BACE is a more appealing target; Aβ production is virtually eliminated in BACE knockout mice, yet (despite a growing list of known substrates for BACE) the mice are viable and apparently healthy [8,16]. Furthermore, raising BACE expression increases plaque load in βAPP-transgenic mice [19]. Discouragingly, finding potent pharmacologic inhibitors of BACE has been challenging, mainly because the large catalytic site of BACE does not avidly bind small molecules [16]. Hence, small molecule gamma secretase inhibitors are potent, but their safety is questionable; BACE inhibitors are more likely to be safe, but the potency of small molecules is low.

In light of the hurdles facing selective beta- and gamma-secretase inhibitors, it is worth seeking other means of manipulating Aβ levels. A variety of agents have been shown to reduce Aβ production, sometimes by indirect or ambiguous mechanisms [8,12]. These compounds, which include such widely used drugs as statins and non-steroidal anti-inflammatories, may present an alternative, and in some cases readily available, approach to lowering Aβ in brain. Whether such agents will alter the progression of AD remains unknown. Furthermore, since Aβ deposition in the transgenic mouse brain differs from that in humans with AD [20], success in rodent intervention studies may not translate into efficacy in humans.

3.2. Impeding Aβ aggregation and toxicity

A second pharmacological tactic is to prevent the aggregation of Aβ into multimeric species (oligomers, protofibrils and fibrils). In vitro, large molecules such as peptides and antibodies can hinder aggregation with high potency; some small molecule inhibitors also have been reported, but protein-protein interactions are notoriously difficult to prevent in vivo using small molecules [21]. In addition, to be most effective, such inhibitors will need to stop protein polymerization prior to the formation of the primary cytotoxic moiety. Compounds that simply obstruct the conversion of protofibrils to fibrils, for example, might prevent senile plaques, but they also could bolster the accumulation of intermediate oligomeric species. Recently, a novel solution to the molecular size-differential problem was reported [22]. Congo red, the classical dye for amyloid, was linked to another small molecule that in turn binds FK506-binding protein (FKBP). This dual-binding agent interposes the comparatively large FKBP between Aβ peptides and potently inhibits aggregation. For safety and bioavailability reasons, Congo red is an unsatisfactory drug candidate. However, if it can be adapted to the in vivo milieu, the ''protein shield'' strategy could be effective in treating AD, as well as other proteopathies.

An alternative to inhibiting the multimerization of Aβ might be to suppress the damaging characteristics of the multimeric species. For instance, Aβ oligomers bind to hippocampal neurons and are linked to deficits in long-term potentiation [15]; furthermore, oligomer-specific antibodies reduce the toxicity of Aβ in vitro [23]. Precisely how oligomers do harm remains uncertain. There is evidence that globular oligomers directly disturb other cellular constituents [24], or that pore-forming assemblies permit the aberrant flux of ions across membranes [25,26]. Impeding the interactions of Aβ oligomers with normal cellular components, or selectively blocking Aβ-ion conduction, thus might forestall neuronal dysfunction in vivo.

Protein production and aggregation occur in the context of a complex cellular and biochemical environment. Aβ oligomers and protofibrils may promote the abnormal polymerization of other proteins, such as tau and α-synuclein [12], although the mechanisms are presently unknown. Finally, numerous conditions can feed into

(and possibly initiate) the proteopathic cascade, such as osmotic, thermal, oxidative, nitrosative and ionic stress [12]. These phenomena are legitimate (and in some cases active) targets for intervention, particularly if directly lowering the production or toxicity of Aβ aggregates proves to be difficult.

3.3. Stimulating Aβ degradation and removal

A third therapeutic option for abrogating the Aβ cascade is to augment the degradation or disposal of Aβ. There are several potential means to this end: (1) enhance enzymatic degradation of Aβ; (2) stimulate the transport of Aβ out of the brain; and (3) engage the immune system in the removal of Aβ aggregates.

3.3.1. Aβ degradation

The Aβ peptide, mainly in its soluble, monomeric form, is degraded by specific endopeptidases, most notably insulin-degrading enzyme (IDE) and neprilysin [27]. Overexpression of IDE and neprilysin in βAPP-transgenic mice lowers Aβ levels and senile plaque load [28]. In addition, an agent that activates α-secretase (the enzyme that cuts βAPP within the Aβ sequence) will reduce the amount of the peptide in brain [16]. Upregulating any of these proteases thus could diminish the accumulation of Aβ and forestall the pathologic process, although oligomers and fibrils tend to be resistant to these enzymes, and the obstacles to selectively increasing enzymatic activity by pharmacological means are considerable.

3.3.2. Aβ transport

Aβ levels in the brain are regulated by a number of processes, including movement across cell membranes and the blood–brain barrier by specific transporter proteins such as the low density lipoprotein receptor-related protein 1 (LRP), receptor for advanced glycation end-products (RAGE) [27,29] and p-glycoprotein (Pgp) [30]. Apolipoproteins E and J also influence the transport and metabolism of Aβ [31]. In theory, upregulating Aβ-efflux proteins such as LRP or Pgp would lower brain levels of Aβ; however, these proteins convey a wide range of substances in different tissues, so the impediments to developing safe and selective Aβ-transport enhancers are no less formidable than for the augmentation of specific proteolytic enzymes.

3.3.3. Aβ immunization

A particularly promising Aβ-removal strategy is to harness the power and specificity of the immune system to eliminate excess Aβ from the brain. Exogenous antibodies to Aβ selectively bind to senile plaques and CAA in vivo [32], and Aβ deposition can be prevented or ameliorated in βAPP-transgenic mice by active immunization with the Aβ peptide [33]. Although the clearance mechanism remains contentious (and probably involves both antibody-mediated phagocytosis and increased efflux of Aβ from brain), ensuing studies in numerous labs have confirmed that diverse anti-Aβ immunization schemes reduce cerebral Aβ burden, as well as behavioral deficits, in βAPP-transgenic mice [34,35].

Based on the success of the approach in mice, and the absence of adverse events in Phase I clinical trials, Elan Pharmaceuticals (in collaboration with Wyeth) initiated Phase II trials of Aβ immunization (AN 1792, active immunization with Aβ42 plus QS-21 adjuvant) in humans with AD. Unexpectedly, approximately 6% of Aβ-vaccinated humans developed clinical evidence of aseptic meningoencephalitis [36], and the trials were halted in early 2002. Despite this setback, the available data from the interrupted trial suggest that AD patients who mounted a significant antibody response showed signs of benefit in some domains ([36,37]; Gilman, 21 July 2004, presentation at the 9th International Conference on Alzheimer's Disease and Related Disorders, Philadelphia, PA, USA). Autopsy findings are currently available for three patients who died approximately one year after the first immunization. In all three cases there is evidence of Aβ clearance from some brain regions [38–40]. However, two of the three cases also had changes consistent with encephalitis, including infiltration of T-lymphocytes (particularly in association with CAA), white matter lesions invaded by macrophages [38,39], and, in one instance, severe small vessel disease and multiple cortical hemorrhages [39]. Furthermore, in a large cohort of trial participants, magnetic resonance imaging (MRI) data demonstrate a greater degree of brain volume loss in antibody responders than in controls during the first year after the onset of treatment (Fox, 21 July 2004 and Nitsch, 22 July 2004, presentations at the Ninth International Conference on Alzheimer's Disease and Related Disorders, Philadelphia, PA, USA). Nitsch reported that the increased loss of brain substance may cease after a year, indicating that this could be a transient phenomenon. Significantly, in the many preclinical studies of immunized mice, there had been no reports of encephalitis or brain shrinkage that would have foreshadowed similar problems in humans. An increased incidence of microhemorrhage, however, has been noted in βAPP-transgenic mice that develop significant CAA [41].

On balance, the results of the clinical trials underscore the potential of Aβ-immunization as a disease-modifying treatment for AD, but only if the serious side-effects can be effectively controlled. Three factors might raise the likelihood of an inflammatory reaction to Aβ immunization. First, the adjuvant used in active immunization protocols can influence the type of cellular response; QS-21, a purified fraction of saponin used in the Elan trials, tends to elicit a T helper cell type 1 (Th1) response, which is associated with cytotoxicity and autoimmunity. In contrast, alum (aluminum salt; currently the only adjuvant that, in certain preparations, is approved for use in humans) promotes a Th2/B-cell response and inhibits autoimmune

disease [42]. A second possible source of inflammation is the Aβ epitope that is targeted. The N-terminal part of Aβ includes a dominant B-cell epitope, and is less likely to induce a cytotoxic T-cell reaction than is full-length Aβ42 [43,44]. Unfortunately, it can be difficult to generate a strong immune response to short peptides in the absence of carrier proteins, a problem that is exacerbated in older subjects whose immune function is often weak. Furthermore, passive immunization of mice with monoclonal antibodies to Aβ3-6 increases the incidence of microvascular hemorrhage [41]. This finding indicates potential liabilities even of N-terminal-directed Aβ antibodies, and also suggests that adjuvant (which is unnecessary for passive immunization) is not required for the induction of hemorrhagic side-effects. Third, the association of focal inflammation with cerebral amyloid angiopathy in two of the three autopsied human cases implies that the presence of Aβ in the vascular wall – CAA – might be particularly likely to elicit an encephalitic response [45].

4. Modeling the side-effects of Aβ immunotherapy

The failure of the mouse experiments to anticipate encephalitis and brain shrinkage argues for testing the safety and efficacy of Aβ immunotherapy in a more biologically relevant model. To this end, we and others have begun to evaluate Aβ-immunization in nonhuman primates [35,46]. Primates are preferred for these studies because of their genetic proximity to humans, the natural occurrence of human-like senile plaques and CAA with age, their behavioral complexity and age-associated cognitive decline, and a relatively large brain size that enables optimal imaging studies [47]. While a type of encephalopathy was recently elicited in Aβ-immunized mice by the administration of pertussis toxin (a virulence factor of *Bordetella pertussis* that stimulates an autoimmune reaction [48]), mice appear to be relatively resistant to the encephalitogenic side-effects of Aβ-immunization.

Our initial Aβ-vaccination experiments established the feasibility of generating a humoral response to aggregated $A\beta^{1-42}$ in middle-aged rhesus monkeys (*Macaca mulatta*) using complete/incomplete Freund's adjuvant. The animals developed antibody titers in excess of 1:1000 as well as elevated levels of plasma Aβ [46]. At 15–20 years of age, the animals were too young to have senile plaques (a process that usually begins in the mid-twenties in rhesus monkeys), so we could not assess plaque clearance. Significantly, none of the animals showed evidence of an inflammatory or autoimmune reaction, suggesting that encephalitis is not an inevitable consequence of Aβ-immunization, even with the use of a strong, Th1-biased adjuvant (Freund's). This finding, in conjunction with the safety and tolerability of immunization shown in the Elan Phase 1 trial, raises the possibility that Aβ deposits must be present in brain to engender an encephalitic reaction in immunized subjects using conventional adjuvants. If so, prophylactic immunization would be safer than therapeutic immunization, a possibility that could be profitably tested in nonhuman primates.

A recent immunization experiment in Caribbean vervet monkeys (*Chlorocebus aethiops*, St. Kitts) further supports the utility of primates for evaluating Aβ vaccination [35]. Four vervet monkeys (16–22 years old) were vaccinated multiple times over a study period of 10 months with a mixture of Aβ40 and Aβ42 plus complete/incomplete Freund's adjuvant. The vervets generated anti-Aβ antibody titers and increased Aβ in plasma, as well as decreased Aβ levels in the CSF, supportive of the hypothesis [49,50] that circulating antibodies to Aβ act as a ''peripheral sink'' to increase the efflux of the peptide from brain. The Aβ antibodies recognized primarily a B-cell epitope in the amino-terminal segment of Aβ. There were small numbers of vascular deposits and no parenchymal (plaque-like) deposits in any of the four immunized monkeys, whereas some plaques (as well as higher levels of Aβ by ELISA) were seen in many of the non-immunized controls. The immunized monkeys also were free of encephalitic and hemorrhagic changes. The results are the first indication in monkeys that Aβ levels in the central nervous system are reduced by Aβ vaccination. It is important to note, however, that the onset of brain Aβ deposition varies considerably in monkeys (and humans) of similar age [47]. Since the immunized vervet monkeys were relatively young (estimated ages of 16{2}, 18 and 22 years), the authors note the possibility that these animals may have had minimal Aβ deposition at the onset of the experiment. If abundant Aβ deposits must be present to provoke immunization-related side-effects, inflammation and microhemorrhage might not be expected in the four immunized monkeys. Vaccination of older animals, with known Aβ loads, would enhance the power of detecting both the beneficial and untoward consequences of the procedure as it relates to the treatment of AD. An even better scenario would be to establish the magnitude of Aβ burden a priori. In this light, employing radiolabeled compounds that label Aβ in vivo [51] could be profitably incorporated into longitudinal primate studies.

Other primate species might have advantages for studying particular aspects of vaccination. One such candidate would be squirrel monkeys (*Saimiri* spp.), which are small (500–1200 g), well-characterized New World primates with a maximum life span of approximately 30 years [47]. Squirrel monkeys begin to form cerebral Aβ lesions around the age of 13 years, and, unlike many other primate species, they have a particular predisposition to develop CAA (Fig. 2B). Since the inflammatory and hemorrhagic changes in immunized humans have been associated with amyloidotic blood vessels (above), vascular Aβ deposition may be an especially strong risk factor for these side-effects. In recent preliminary studies, we determined that aged squirrel monkeys develop a robust immune response

to aggregated, recombinant Aβ42 using alum as the adjuvant. In one case, there were multiple cortical microhemorrhages and focal inflammation associated with CAA, supporting the aged squirrel monkey model for assessing the safety and efficacy of Aβ immunotherapy.

5. Conclusions

An encouraging concept that has emerged from contemporary research on neurodegenerative diseases is that the complex pathology of end-stage AD can be traced back to a relatively simple, and potentially tractable, process: the ordered aggregation of Aβ in brain. The means whereby Aβ aggregation disrupts brain function is still debated, but a wealth of studies now indicate that simply reducing the amount of Aβ will impede the Aβ cascade sufficiently to be of therapeutic utility. There are many potential ways to achieve this goal, but the most direct and therapeutically feasible routes at present are to: (1) inhibit the enzymes that free Aβ from its parental protein; (2) promote cellular Aβ degradation and efflux; or (3) stimulate immune-mediated removal of Aβ. It is possible that combination approaches to AD therapy will be needed to achieve full efficacy, including disease-modifying and palliative treatments. One therapy in particular that has shown extraordinary effects on brain pathology and behavior in mice is Aβ-immunization, and limited data from the Elan/Wyeth therapeutic trial suggest possible benefits in humans. The potential of the approach is currently limited by the emergence of encephalitic side-effects and an unexpected acceleration of brain shrinkage, neither of which was predicted from preclinical studies in transgenic mice. Testing Aβ-immunotherapy in aged nonhuman primates, which naturally develop β-amyloid lesions in brain, can augment our understanding of the adverse events in a biologically optimal model, and thereby speed the safe application of this promising therapy to humans with Alzheimer's disease.

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References

[1] Hauw J-J, Duyckaerts C. Alzheimer's Disease. In: Duckett S, De La Torre JC, editors. Pathology of the Aging Human Nervous System. New York: Oxford University Press; 2001. p. 207–63.
[2] Cummings JL. Alzheimer's disease. N Engl J Med 2004;351(1):56–67.
[3] Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002; 297(5580):353–6.
[4] Lee VM, Goedert M, Trojanowski JQ. Neurodegenerative tauopathies. Annu Rev Neurosci 2001;24:1121–59.
[5] Fox NC, Schott JM. Imaging cerebral atrophy: normal ageing to Alzheimer's disease. Lancet 2004;363(9406):392–4.
[6] Mesulam M. The cholinergic lesion of Alzheimer's disease: pivotal factor or side show? Learn Membr 2004;11(1):43–9.
[7] Bartus RT. On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. Exp Neurol 2000;163(2):495–529.
[8] Scarpini E, Scheltens P, Feldman H. Treatment of Alzheimer's disease: current status and new perspectives. Lancet Neurol 2003;2(9):539–47.
[9] Rogawski MA. What is the rationale for new treatment strategies in Alzheimer's disease? CNS Spectr 2004;9(7 Suppl. 5):6–12.
[10] Knopman D. Pharmacotherapy for Alzheimer's disease: 2002. Clin Neuropharmacol 2003;26(2):93–101.
[11] Marjaux E, Hartmann D, De Strooper B. Presenilins in memory, Alzheimer's disease, and therapy. Neuron 2004;42(2):189–92.
[12] Walker LC, LeVine III H. Proteopathy: the next therapeutic frontier? Curr Opin Investig Drugs 2002;3(5):782–7.
[13] Oddo S, Billings L, Kesslak JP, Cribbs DH, LaFerla FM. Abeta immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome. Neuron 2004;43(3):321–32.
[14] Klein WL. Abeta toxicity in Alzheimer's disease: globular oligomers (ADDLs) as new vaccine and drug targets. Neurochem Int 2002;41(5): 345–52.
[15] Walsh DM, Selkoe DJ. Deciphering the molecular basis of memory failure in Alzheimer's disease. Neuron 2004;44(1):181–93.
[16] Dewachter I, Van Leuven F. Secretases as targets for the treatment of Alzheimer's disease: the prospects. Lancet Neurol 2002;1(7):409–16.
[17] Yu H, Saura CA, Choi SY, Sun LD, Yang X, Handler M, et al. APP processing and synaptic plasticity in presenilin-1 conditional knockout mice. Neuron 2001;31(5):713–26.
[18] Saura CA, Choi SY, Beglopoulos V, Malkani S, Zhang D, Shankaranarayana Rao BS, et al. Loss of presenilin function causes impairments of memory and synaptic plasticity followed by age-dependent neurodegeneration. Neuron 2004;42(1):23–36.
[19] Willem M, Dewachter I, Smyth N, Van Dooren T, Borghgraef P, Haass C, et al. Beta-site amyloid precursor protein cleaving enzyme 1 increases amyloid deposition in brain parenchyma but reduces cerebrovascular amyloid angiopathy in aging BACE × APP[V717I] double-transgenic mice. Am J Pathol 2004;165(5):1621–31.
[20] Kalback W, Watson MD, Kokjohn TA, Kuo YM, Weiss N, Luehrs DC, et al. APP transgenic mice Tg2576 accumulate Abeta peptides that are distinct from the chemically modified and insoluble peptides deposited in Alzheimer's disease senile plaques. Biochemistry 2002;41(3):922–8.
[21] LeVine III H. Challenges of targeting A beta fibrillogenesis and other protein folding disorders. Amyloid 2003;10(3):133–5.
[22] Gestwicki JE, Crabtree GR, Graef IA. Harnessing chaperones to generate small-molecule inhibitors of amyloid beta aggregation. Science 2004;306(5697):865–9.
[23] Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, et al. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 2003;300(5618):486–9.
[24] Bahr BA, Hoffman KB, Yang AJ, Hess US, Glabe CG, Lynch G. Amyloid beta protein is internalized selectively by hippocampal field CA1 and causes neurons to accumulate amyloidogenic carboxyterminal fragments of the amyloid precursor protein. J Comp Neurol 1998;397(1):139–47.
[25] Lashuel HA, Hartley D, Petre BM, Walz T, Lansbury Jr PT. Neurodegenerative disease: amyloid pores from pathogenic mutations. Nature 2002;418(6895):291.

[26] Kayed R, Sokolov Y, Edmonds B, McIntire TM, Milton SC, Hall JE, et al. Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. J Biol Chem 2004;279(45):46363–6.
[27] Tanzi RE, Moir RD, Wagner SL. Clearance of Alzheimer's A beta peptide: the many roads to perdition. Neuron 2004;43(5):605–8.
[28] Leissring MA, Farris W, Chang AY, Walsh DM, Wu X, Sun X, et al. Enhanced proteolysis of beta-amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death. Neuron 2003;40(6):1087–93.
[29] Deane R, Wu Z, Zlokovic BV. RAGE (yin) versus LRP (yang) balance regulates Alzheimer amyloid beta-peptide clearance through transport across the blood-brain barrier. Stroke 2004;35(11 Suppl. 1): 2628–31.
[30] Vogelgesang S, Warzok RW, Cascorbi I, Kunert-Keil C, Schroeder E, Kroemer HK, et al. The role of *p*-glycoprotein in cerebral amyloid angiopathy; Implications for the early pathogenesis of Alzheimer's disease. Curr Alzheimer Res 2004;1(2):121–5.
[31] DeMattos RB, Cirrito JR, Parsadanian M, May PC, O'Dell MA, Taylor JW, et al. ApoE and clusterin cooperatively suppress abeta levels and deposition. evidence that ApoE regulates extracellular Abeta metabolism in vivo. Neuron 2004;41(2):193–202.
[32] Walker LC, Price DL, Voytko ML, Schenk DB. Labeling of cerebral amyloid in vivo with a monoclonal antibody. J Neuropathol Exp Neurol 1994;53(4):377–83.
[33] Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 1999;400(6740):173–7.
[34] Dodel RC, Hampel H, Du Y. Immunotherapy for Alzheimer's disease. Lancet Neurol 2003;2(4):215–20.
[35] Lemere CA, Beierschmitt A, Iglesias M, Spooner ET, Bloom JK, Leverone JF, et al. Alzheimer's disease abeta vaccine reduces central nervous system abeta levels in a non-human primate, the Caribbean vervet. Am J Pathol 2004;165(1):283–97.
[36] Orgogozo JM, Gilman S, Dartigues JF, Laurent B, Puel M, Kirby LC, et al. Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. Neurology 2003;61(1):46–54.
[37] Hock C, Konietzko U, Streffer JR, Tracy J, Signorell A, Muller-Tillmanns B, et al. Antibodies against beta-amyloid slow cognitive decline in Alzheimer's disease. Neuron 2003;38(4):547–54.
[38] Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. Nat Med 2003;9(4):448–52.
[39] Ferrer I, Boada Rovira M, Sanchez Guerra ML, Rey MJ, Costa-Jussa F. Neuropathology and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease. Brain Pathol 2004;14(1):11–20.
[40] Masliah E, Hansen L, Adame A, Crews L, Bard F, Lee C, et al. Abeta vaccination effects on plaque pathology in the absence of encephalitis in Alzheimer disease. Neurology 2005;64(1):129–31.
[41] Pfeifer M, Boncristiano S, Bondolfi L, Stalder A, Deller T, Staufenbiel M, et al. Cerebral hemorrhage after passive anti-Abeta immunotherapy. Science 2002;298(5597):1379.
[42] Cribbs DH, Ghochikyan A, Vasilevko V, Tran M, Petrushina I, Sadzikava N, et al. Adjuvant-dependent modulation of Th1 and Th2 responses to immunization with beta-amyloid. Int Immunol 2003;15(4):505–14.
[43] McLaurin J, Cecal R, Kierstead ME, Tian X, Phinney AL, Manea M, et al. Therapeutically effective antibodies against amyloid-beta peptide target amyloid-beta residues 4-10 and inhibit cytotoxicity and fibrillogenesis. Nat Med 2002;8(11):1263–9.
[44] Bard F, Barbour R, Cannon C, Carretto R, Fox M, Games D, et al. Epitope and isotype specificities of antibodies to beta-amyloid peptide for protection against Alzheimer's disease-like neuropathology. Proc Natl Acad Sci USA 2003;100(4):2023–8.
[45] Gandy S, Walker L. Toward modeling hemorrhagic and encephalitic complications of Alzheimer amyloid-beta vaccination in nonhuman primates. Curr Opin Immunol 2004;16(5):607–15.
[46] Gandy S, DeMattos RB, Lemere CA, Heppner FL, Leverone J, Aguzzi A, et al. Alzheimer A beta vaccination of rhesus monkeys (*Macaca mulatta*). Alzheimer Dis Assoc Disord 2004;18(1):44–6.
[47] Walker LC, Cork LC. The neurobiology of aging in nonhuman primates. In: Terry RD, Katzman R, Bick KL, Sisodia SS, editors. Alzheimer Disease. Philadelphia: Lippincott Williams and Wilkins; 1999. p. 233–43.
[48] Furlan R, Brambilla E, Sanvito F, Roccatagliata L, Olivieri S, Bergami A, et al. Vaccination with amyloid-beta peptide induces autoimmune encephalomyelitis in C57/BL6 mice. Brain 2003;126:285–91.
[49] DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM. Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. Proc Natl Acad Sci USA 2001;98(15):8850–5 (Epub 2001 July 03).
[50] Matsuoka Y, Saito M, LaFrancois J, Saito M, Gaynor K, Olm V, et al. Novel therapeutic approach for the treatment of Alzheimer's disease by peripheral administration of agents with an affinity to beta-amyloid. J Neurosci 2003;23(1):29–33.
[51] Mathis CA, Wang Y, Klunk WE. Imaging beta-amyloid plaques and neurofibrillary tangles in the aging human brain. Curr Pharm Des 2004;10(13):1469–92.