

# Amyloid- $\beta$ immunisation for Alzheimer's disease



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Alzheimer's disease is the main cause of dementia in elderly people and is becoming an ever greater problem as societies worldwide age. Treatments that stop or at least effectively modify disease course do not yet exist. In Alzheimer's disease, the conversion of the amyloid- $\beta$  peptide (A $\beta$ ) from a physiological water-soluble monomeric form into neurotoxic oligomeric and fibrillar forms rich in stable  $\beta$ -sheet conformations is an important event. The most toxic forms of A $\beta$  are thought to be oligomers, and dimers might be the smallest neurotoxic species. Numerous immunological approaches that prevent the conversion of the normal precursor protein into pathological forms or that accelerate clearance are in development. More than ten new approaches to active and passive immunotherapy are under investigation in clinical trials with the aim of producing safe methods for immunological therapy and prevention. A delicate balance between immunological clearance of an endogenous protein with acquired toxic properties and the induction of an autoimmune reaction must be found.

## Introduction

Alzheimer's disease is one of several disorders associated with conformational protein aggregations with overlap in pathological mechanism; others include prion, Parkinson's, and Huntington's diseases.<sup>1</sup> The basic pathological mechanism in these disorders is a conformational change of a normally expressed protein. In the case of Alzheimer's disease, both water-soluble amyloid- $\beta$  peptides (A $\beta$ ) and tau proteins form  $\beta$ -sheet toxic forms. Deposits of A $\beta$  form neuritic plaques and cerebral amyloid angiopathy, and hyperphosphorylated tau aggregates within neurons as paired helical filaments in neurofibrillary tangles.<sup>2</sup>

Aggregation and structural conversion occurs without changes to the amino-acid sequence of the proteins and results in a highly complex dynamic equilibrium of fibrillation intermediates in which early oligomeric species can act as seeds for fibrillation. A $\beta$  is a 40–43 residue peptide that is a cleavage product of the amyloid precursor protein.<sup>3</sup> Missense mutations in the gene encoding this protein, *APP*, or in the presenilin genes *PRES1* and *PRES2* can cause early-onset, familial forms of Alzheimer's disease; however, the most common form of Alzheimer's disease is sporadic and late-onset.

Derivatives of amyloid precursor protein, including water-soluble A $\beta$  peptides, are present in most physiological fluids including plasma and CSF.<sup>1</sup> In Alzheimer's disease, aggregation of water-soluble, monomeric A $\beta$  peptides into oligomeric forms is associated with conformational changes and neurotoxicity, including the impairment of long-term potentiation and accelerated formation of neurofibrillary tangles.<sup>1,4</sup> Whether A $\beta$  peptide aggregation into oligomers and deposited fibrils are steps in the same pathway or independent pathways is unknown.

## Conformational change in soluble A $\beta$

Several proteins can promote the conformational transformation of disease-specific proteins and stabilise their abnormal structure; in Alzheimer's disease, these include apolipoprotein E (APOE), especially its  $\epsilon 4$  isoform,<sup>5</sup>  $\alpha 1$ -antichymotrypsin,<sup>6</sup> and C1q complement

factor.<sup>7,8</sup> These proteins greatly increase formation of A $\beta$  fibrils from water-soluble A $\beta$ .<sup>5,6</sup> These pathological chaperone proteins have been found histologically and biochemically in association with fibrillar A $\beta$  deposits<sup>9</sup> but not in preamyloid aggregates, which are not associated with neuronal loss.<sup>10</sup> An important event in the pathomechanism of Alzheimer's disease is thought to be the reaching of a crucial concentration of water-soluble A $\beta$  or chaperone proteins in the brain, at which point conformational change occurs, leading to formation of aggregates, initiating a neurodegenerative cascade. In sporadic Alzheimer's disease, this crucial concentration might be reached because of any combination of age-associated overproduction of A $\beta$ , impaired clearance from the brain, or influx of circulatory A $\beta$  into the CNS.<sup>11</sup>

## A $\beta$ in familial and sporadic AD

Accumulation of toxic, aggregated forms of A $\beta$  seem crucial in the pathogenesis of familial forms of Alzheimer's disease.<sup>12</sup> Some inherited forms are linked to mutations in *APP*, *PRES1*, or *PRES2* that affect the processing of amyloid precursor protein, leading to overproduction of soluble A $\beta$  or production of aggregation-prone forms, such as A $\beta_{1-42}$ .<sup>13</sup> Down's syndrome, in which there is an extra copy of *APP* because of trisomy 21, is associated with Alzheimer's disease pathology at a very early age.<sup>14</sup> In transgenic and other models of coexpressed A $\beta$  and tau, A $\beta$  oligomer formation precedes and accentuates tau-related pathology, which is consistent with the hypothesis that formation of neurofibrillary tangles is downstream of A $\beta$  aggregation.<sup>15–17</sup> In transgenic mouse models of mutant *APP* overexpression without tau pathology, therapeutic prevention or removal of A $\beta$  is associated with cognitive benefits.<sup>18–21</sup> Importantly, in transgenic mouse models of mutant *APP* and tau overexpression, prevention of A $\beta$  pathology leads to amelioration of both cognitive deficits and tau-related pathology.<sup>22–24</sup>

Evidence linking A $\beta$  to sporadic Alzheimer's disease is less extensive. Many studies show a weak correlation between A $\beta$  deposits and cognitive status,<sup>25</sup> and some

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show that cognitively healthy elderly people can have substantial amyloid burden.<sup>26,27</sup> Specific evidence for a central role of A $\beta$  in sporadic disease includes an association between biochemically extracted A $\beta$  peptides from brains of people with cognitive decline (by contrast with studies of histologically measured amyloid deposits).<sup>28</sup> Furthermore, A $\beta$  extracted from the brains of patients with sporadic disease induces amyloid deposits when injected into transgenic mice,<sup>29</sup> and directly isolated A $\beta$  dimers impair synaptic structure and function.<sup>30</sup> Although the amyloid-cascade hypothesis is the dominant theory, some researchers suggest that A $\beta$  accumulation is a marker for the presence of disease, rather than central to pathogenesis.<sup>25,31</sup> The ultimate test of this theory will be when treatments that prevent or remove A $\beta$  aggregates are fully tested in human beings.

### Mechanisms of A $\beta$ -directed immunomodulation

Vaccination was the first treatment approach to have genuine effect on the Alzheimer's disease process, at least in animal models. Vaccination of transgenic mice with A $\beta_{1-42}$  or an A $\beta$  homologue and Freund's adjuvant prevented A $\beta$  deposition and, as a consequence, prevented behavioural impairments related to A $\beta$  deposition.<sup>18-21,32,33</sup> Peripheral injections of monoclonal antibodies against A $\beta$  have similar effects on A $\beta$  load and behaviour, indicating that the therapeutic effect of the vaccine is based primarily on the eliciting of a humoral response.<sup>34,35</sup>

A $\beta$  vaccination could elicit a humoral response by at least six possible mechanisms that are not mutually exclusive (figure).<sup>36-38</sup> A $\beta$  antibodies that are selective for specific conformations might target A $\beta$  deposits in the

brain leading to direct disassembly.<sup>39</sup> Some antibodies are able to dissolve A $\beta$  fibrils in vitro, preventing reassembly and inhibiting toxicity;<sup>40-42</sup> in the brain, these antibodies might also activate microglia to clear plaques by eliciting Fc-mediated phagocytosis.<sup>34</sup> The Fc portion of A $\beta$  antibodies is not necessary for A $\beta$  clearance, and APP transgenic mice crossed with FcR $\gamma$ -chain knockout mice, which have complete impairment of phagocytosis of A $\beta$  immune complexes via FcR, respond to vaccination in much the same way as FcR-sufficient mice.<sup>43</sup> Furthermore, direct application of F(ab')<sub>2</sub> fragments of A $\beta$  antibodies can clear amyloid deposits in vivo.<sup>44</sup> These findings, along with observations of microglial activation after passive immunisation,<sup>45</sup> suggest an Fc-independent mechanism of phagocytosis and degradation.<sup>38</sup> The fourth mechanism by which antibodies could prevent A $\beta$  deposition is the creation of a peripheral-sink effect, in which the removal of excess circulatory soluble A $\beta$  draws soluble A $\beta$  from the brain.<sup>19,32,35,46</sup> The potential importance of this mechanism is illustrated by active immunisation experiments in which a non-toxic, non-fibrillogenic modified A $\beta$  peptide was used with alum as an adjuvant that primarily stimulates a humoral immune response.<sup>32</sup> This active immunisation protocol only elicited an IgM immune response to A $\beta$ . Because of its larger size, IgM crosses the blood-brain barrier much less than does IgG, but vaccinated mice had both reduction of amyloid burden and cognitive improvement. These effects were presumably mediated mainly via a peripheral-sink mechanism. IgM might also function by hydrolysing A $\beta$ . Antibodies might also neutralise neurotoxic oligomers.<sup>47</sup>

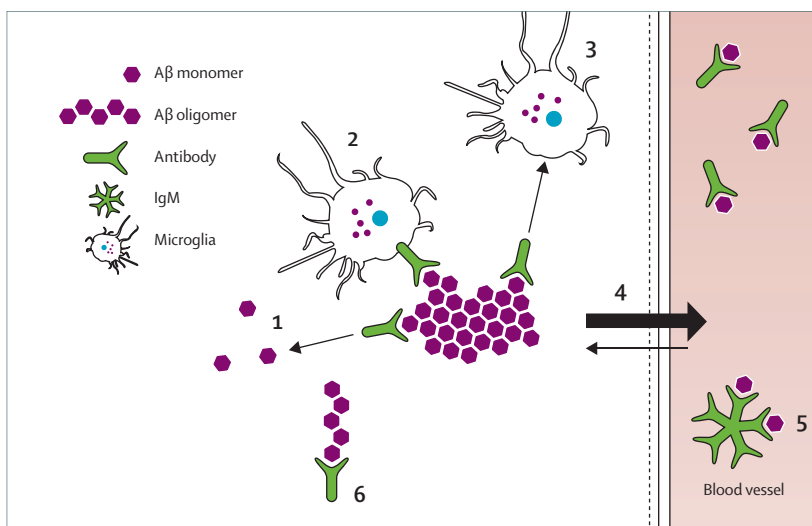
### A $\beta$ vaccination in human beings

#### Active immunisation

The striking biological effect of vaccination in preclinical testing and the apparent lack of side-effects in transgenic mice encouraged the launch of clinical trials with AN1792, a vaccine that contained preaggregated A $\beta_{1-42}$  and QS21. Because QS21 strongly induces Th1 lymphocytes, this vaccine design aimed to induce a strong cell-mediated immune response.<sup>48</sup>

The initial UK trial in 80 patients with mild to moderate Alzheimer's disease<sup>49</sup> was designed to assess the antigenicity and toxicity of multiple-dose immunisation. 53% of patients developed an anti-A $\beta$  humoral response. During the later stages of the phase I trial, the emulsifier polysorbate 80 was added causing a shift from a predominantly Th2 response to a proinflammatory Th1 response.<sup>50</sup> In the subsequent phase II trial, 372 patients were enrolled, with 300 receiving the aggregated A $\beta_{1-42}$  (AN1792) with QS21 in the polysorbate 80 formulation. This trial was stopped early when 18 (6%) of 298 of vaccinated patients had symptoms of acute meningococcal phalitis.<sup>48,51,52</sup>

Autopsy investigations of a few participants showed clearance of parenchymal plaques, similar to that in the



**Figure: Potential mechanisms of immunomodulation for amyloid- $\beta$  related pathology**  
Direct disassembly of plaques by conformation-selective antibodies (1); antibody mediated activation of microglial cells (2); non-Fc mediated activation of microglia (3); creation of peripheral sink by clearance of circulating amyloid  $\beta$  (4); IgM-mediated hydrolysis (5); neutralisation of oligomer toxicity (6). These mechanisms are not mutually exclusive. More than one mechanism could play a part at any give time, with different mechanisms potentially having a role at different stages of disease.

animal studies, confirming the validity of this approach for amyloid clearance in human beings.<sup>52-57</sup> Extensive areas of cerebral cortex were devoid of plaques, with residual plaques having a “moth-eaten” appearance or lasting as “naked” dense cores. Amyloid clearance in most cases was associated with microglia that showed A $\beta$  immunoreactivity, suggesting phagocytosis. Additional notable features were the persistence of amyloid in cerebral vessels and unaltered tau-immunoreactive neurofibrillary tangles and neuropil threads in regions of cerebral cortex where plaque clearance had apparently occurred.<sup>55-57</sup> Some patients also had a deleterious T-cell reaction surrounding some cerebral vessels, suggestive of an excessive Th1 immune response.

Immune reaction triggered by AN1792 seemed to be a double-edge sword: the benefits of humoral response against A $\beta$  were overshadowed in some individuals by a detrimental T-cell-mediated inflammatory response.<sup>52,58</sup> Not all patients who received AN1792 responded with antibody production. Most had a humoral response, modest but statistically significant improvement on some cognitive testing scales compared with baseline, and a slowed rate of disease progression compared with patients who did not form antibodies.<sup>49,59</sup> Follow-up data from the Zurich cohort, a subset of the AN1792 trial,<sup>59,60</sup> indicate that the vaccination approach might be beneficial for patients with Alzheimer’s disease. And immune responders with high antibody titres in the multicentre cohort scored significantly better in composite scores of memory functions than did non-responders or patients who received placebo.<sup>50</sup>

Despite the apparent success in amyloid clearance indicated by autopsy data, clinical cognitive benefits in the active vaccination group compared with placebo were very modest.<sup>61</sup> This finding could be related to the small decline in cognitive function in the placebo group,<sup>61,62</sup> although a similar result in a vaccination study in dogs might suggest otherwise.<sup>63</sup> Elderly dogs are a natural

model of A $\beta$  amyloidosis, because the canine APP protein sequence is about 98% similar to human APP. In a 2-year study, cortical A $\beta$  immunoreactivity decreased by about 80% in most areas; however, this decline was not associated with any improvements in complex learning, spatial memory, or attention.<sup>63</sup> As in the human data, maintenance of executive function was recorded in the dogs. These data suggest that active vaccination needs to start before the development of clinically significant Alzheimer’s disease-related pathology.

Persistence of tau-related pathology in cortical areas cleared of amyloid indicates that intervention might have been too late. This idea is supported by recent data from the follow-up of the 80 patients in the phase I AN1792 trial, eight of whom had an autopsy.<sup>62</sup> Despite evidence of very significant amyloid plaque removal (which was related to antibody titre) in six patients, in the overall group there was no evidence of improved survival or lengthening of time to severe dementia.<sup>62</sup> If immunisation begins early, A $\beta$ -lowering might prevent formation of neurofibrillary tangles, which seems to be a result of A $\beta$ -related toxicity,<sup>15,27</sup> and thus vaccination could provide better cognitive benefits than it has in trials to date.

In transgenic mice, antibodies cleared both A $\beta$  and early, but not late, forms of hyperphosphorylated tau aggregations.<sup>64</sup> Therefore, A $\beta$  immunotherapy could prevent formation of new tangles without affecting numbers or morphology of those already formed. Several trials of active human immunisation are underway (table).<sup>49,50,61,65</sup>

The cause of toxicity in 6% of patients in the AN1792 trial is unknown; however, cytotoxic T-cell reactions surrounding some cerebral vessels as seen at autopsy suggest an excessive Th1-mediated response.<sup>66</sup> The likely involvement of excess cell-mediated response in toxicity was supported by analysis of participants’ peripheral-blood mononuclear cells. When stimulated *in vitro* with A $\beta$ , cells from most participants who showed a response

	Phase	Intervention	Primary outcomes	Size	Duration
<b>Active immunisation</b>					
NCT00498602	Phase II	ACC-001+Q521 vs ACC-001 vs placebo	Safety, tolerability	228	Nov, 2007, to March, 2012
NCT00411580	Phase I	CAD106	Safety, tolerability	60	June, 2005, to April, 2008
NCT00464334	Phase I	V950	Safety	70	April, 2007, to Sept, 2011
<b>Passive immunisation</b>					
NCT00575055	Phase III	Bapineuzumab	Cognitive, functional	800	Dec, 2007, to Dec, 2010
NCT00329082	Phase II	LY2062430	Safety, tolerability	25	May, 2006, to May, 2008
NCT00299988	Phase II	Intravenous immunoglobulin	ADAS-cog, ADAS-CGIC	24	Start Feb, 2006; ongoing but recruitment complete
NCT00455000	Phase I	PF-04360365	Safety, tolerability, pharmacokinetics	36	March, 2007, to June, 2008
NCT00531804	Phase I	R1450	Adverse events, laboratory measures, vital signs	80	Dec, 2006, to Jan, 2009

ADAS-cog/CGIC=Alzheimer’s disease assessment score cognitive scale/clinician’s global impression of change.

**Table: Current randomised, double-blind, parallel-assignment studies of immunotherapy in Alzheimer’s disease**

produced interleukin 2 and interferon  $\gamma$  indicative of a class II (CD4+) Th1-type response.<sup>50</sup> Hence, a redesigned vaccine will need to avoid this cell-mediated response by avoiding stimulation of Th1 lymphocytes so that the vaccine could potentially elicit a purely humoral response; by using non-toxic and non-fibrillogenic A $\beta$  homologous peptides, so that the immunogen does not produce direct toxicity; and by enhancing the peripheral-sink effect rather than central action.

### Passive immunisation

Passive transfer of exogenous monoclonal A $\beta$  antibodies seems the easiest way to provide antibodies without eliciting Th1-mediated autoimmunity. Transgenic mice treated this way had significant decreases in A $\beta$  concentration and cognitive benefit.<sup>34,35</sup> Major challenges of this approach are high costs, blood–brain barrier penetration, microhaemorrhage, off-target cross-reactivity, and loss of the antibody to a peripheral sink. Nevertheless, at least four clinical trials for passive immunisation with various approaches are underway (table).

The most advanced trial is of bapineuzumab: Elan/Wyeth recently initiated a phase III trial and released preliminary analysis of the phase II results.<sup>67</sup> The phase II trial was a randomised, double-blind, placebo-controlled trial testing three doses of a humanised A $\beta$  antibody in 240 participants. In each of the escalating doses of the antibody, about 32 patients received active agent and 28 received placebo. Although the study did not attain statistical significance on the primary efficacy endpoints in the whole study population over the 18-month trial period, in the subgroup of participants who did not have the *APOE*  $\epsilon$ 4 allele clinically significant benefits were recorded on several scales, including the mini-mental state examination and the Alzheimer's disease assessment scale battery. Furthermore, in the same subgroup, MRI showed less loss of brain volume in treated patients than in control patients. These findings suggest that this form of therapy might be effective. However, some patients in the treatment group, but not in the control group, had vasogenic oedema, a serious adverse event. In another study, intravenous immunoglobulin containing antibodies against A $\beta$  affected A $\beta$  plasma concentrations in patients,<sup>65</sup> and this approach is undergoing further studies. Alternative approaches for passive immunisation less likely to be associated with toxicity include the use of Fv fragments or mimetics of the active antibody-binding site.<sup>68</sup>

Microhaemorrhage is a particular concern in studies of passive immunisation. The mechanism of microhaemorrhage is probably related to vascular amyloid deposits (conophilic amyloid angiopathy), which cause degeneration of smooth muscle cells and weakening of the blood-vessel wall. Congophilic amyloid angiopathy is present in nearly all patients with Alzheimer's disease and is severe in about 20%.<sup>69</sup> Furthermore, amyloid

angiopathy is present in about 33% of cognitively healthy elderly control populations.<sup>70</sup> Several reports have shown an increase in microhaemorrhages in mouse models of Alzheimer's disease after passive intraperitoneal immunisation with different monoclonal antibodies with high affinity for A $\beta$  plaques and congophilic amyloid angiopathy.<sup>71–73</sup> Microhaemorrhages after active immunisation in a transgenic mouse model were noted in one study.<sup>74</sup> In such models, A $\beta$  antibodies both prevent the deposition of vascular amyloid and remove aggregates, thus contributing to vascular repair. However, early autopsies from the AN1792 trial indicated no clearance of vascular amyloid; one patient had numerous cortical bleeds, which are typically rare in patients with Alzheimer's disease and might, therefore, have been related to immunisation.<sup>54</sup> The need for vascular repair and regeneration during A $\beta$  immunotherapy is another argument for early treatment and subtle clearance over a long time period.

### Alternative strategies for vaccination

Understanding the antigenic profile of A $\beta$  peptide allows engineering of modifications that favour a humoral response and reduce the potential for a Th1-mediated response. This approach has been termed altered peptide ligands. Computer models have predicted that A $\beta$ <sub>1–42</sub> has one major antibody-binding site located on its N-terminus and two major T-cell epitopes located at the central and C-terminal hydrophobic regions encompassing residues 17–21 and 29–42, respectively.<sup>75,76</sup> Therefore, elimination or modification of these sites provides a double gain by eliminating toxicity and the potential for T-cell stimulation. Sigurdsson and colleagues<sup>32</sup> immunised transgenic mice with K6A $\beta$ <sub>1–30</sub>[E<sub>18</sub>E<sub>19</sub>], a non-toxic A $\beta$ -homologous peptide in which the first T-cell epitope was modified and the second removed. Polyamino-acid chains coupled to its N-terminus were designed to increase the immunogenicity and solubility of the peptide. The mice produced mainly IgM class antibodies; IgG was absent or present at low titres and showed behavioural improvement and a partial clearance of A $\beta$  deposits.<sup>32,33</sup> One of the advantages of this design is that IgM, with a molecular weight of 900 kDa, penetrates the blood–brain barrier to a lesser degree than IgG and is therefore less likely to be associated with immune reaction in the brain. As with passive immunisation, this type of vaccine focuses its mechanism of action on the peripheral sink. Furthermore, the IgM response is reversible because it is T-cell independent; hence, memory T cells that could maintain the immune response are not generated. Therefore, this vaccination method might be safer than typical active immunisation.

Mucosal vaccination is 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 gastrointestinal tract. This type of response produces primarily secretory IgA antibodies,

but when the antigen is coadministered with adjuvants such as cholera toxin subunit B or heat-labile *Escherichia coli* enterotoxin, substantial serum IgG titres can be achieved.<sup>77,78</sup> Immunisation of transgenic mice with A $\beta$  as an antigen reduces amyloid burden.<sup>78,79</sup> Mucosal immunisation is highly effective for prion infection.<sup>80</sup> The great potential advantage of mucosal immunisation is a more limited humoral immune response with little or no cell-mediated immunity.

Another potentially attractive means to produce a robust humoral response that is mainly Th2 is with the use of DNA epitope vaccines.<sup>81</sup> One such prototype vaccine that consisted of three copies of the B-cell epitope (A $\beta$ <sub>1-11</sub>), a non-self Th-cell epitope (PADRE), and a macrophage-derived chemokine (MDC/CCL22) as an adjuvant to drive a Th2 response, is highly effective in a mouse model of Alzheimer's disease.<sup>81</sup> This type of technology has received substantial interest because of the ease of selectively designing these vaccines to elicit specific immune responses.

Stimulation of innate immunity rather than adaptive immune responses of T cells and B cells can produce an immune response to a self protein. Such stimulation can be achieved by direct activation of microglia via Toll-like receptors and might help avoid toxicity. Toll-like receptors are a family of innate immune mediators expressed by various immune and non-immune cells.<sup>82</sup> Results of studies in prion diseases suggest that stimulation of Toll-like receptor 9 with CpG oligodeoxynucleotides is an attractive candidate for Alzheimer's disease prevention and treatment.<sup>83,84</sup> The potential therapeutic importance of the innate immune system to A $\beta$  pathology is illustrated by reductions in the amyloid burden of up to 90% in transgenic mice in which the TGF- $\beta$ -Smad2/3 signalling pathway was blocked in innate immune cells.<sup>85</sup>

### Future directions

Numerous studies in animal models of Alzheimer's disease suggest that vaccination can prevent the devastating effects of this prevalent disorder. However, a balance must be achieved between effective prevention and clearance of amyloid deposits and the induction of autoimmunity. Initial human trials of active vaccination did not achieve this balance, and a minority of patients developed encephalitis because of excessive Th1-cell responses. New active vaccines are being engineered to drive Th2 or Th3 responses or stimulate innate immunity. Apart from overcoming toxicity, effective vaccines need to provide greater benefit for cognition than those tested so far. This benefit is likely to rely on identification of preclinical amyloidosis with imaging techniques and other interventions, such as cognitive rehabilitation, that might restore neuronal health after removal of toxicity. With the multiple approaches to amyloid prevention in development, we believe that the near future will produce a final answer on whether the amyloid-cascade hypothesis is correct.

### Search strategy and selection criteria

References for this review were identified by searches of Pubmed from January 1972 to July 2008 with the terms "vaccine", "vaccination", "Alzheimer's disease", "immunomodulation", "immunotherapy", "clinical trials", "amyloid", and "amyloid  $\beta$ ". Only papers published in English were reviewed.

### Contributors

Both authors contributed to the data search and writing of the Review. TW wrote a draft and UK provided revisions.

### Conflicts of interest

TW has no conflicts of interest. UK is co-author on a patent for the tissue amyloid plaque immunoreactivity (TAPIR) assay, held by the University of Zurich.

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