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Alzheimer's disease: is a vaccine possible?

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Abstract

The cause of Alzheimer's disease is still unknown, but the disease is distinctively characterized by the accumulation of β-amyloid plaques and neurofibrillary tangles in the brain. These features have become the primary focus of much of the research looking for new treatments for the disease, including immunotherapy and vaccines targeting β-amyloid in the brain. Adverse effects observed in a clinical trial based on the β-amyloid protein were attributed to the presence of the target antigen and emphasized the relevance of finding safer antigen candidates for active immunization. For this kind of approach, different vaccine formulations using DNA, peptide, and heterologous prime-boost immunization regimens have been proposed. Promising results are expected from different vaccine candidates encompassing B-cell epitopes of the β-amyloid protein. In addition, recent results indicate that targeting another protein involved in the etiology of the disease has opened new perspectives for the effective prevention of the illness. Collectively, the evidence indicates that the idea of finding an effective vaccine for the control of Alzheimer's disease, although not without challenges, is a possibility.

Key words: Alzheimer's disease; β-amyloid; Vaccine; Active immunization

Introduction

Alzheimer's disease (AD), the most common form of dementia in elderly people, is characterized by a progressive decline of brain functions, including memory, language, spatial orientation, and behavior, finally resulting in death. This disease was first described by the German physician Alois Alzheimer in 1906 (published in 1907), based on his studies of a 51-year-old female patient who presented symptoms of dementia, beginning with changes in personality and progressive memory loss, and a life prognosis of 4 to 5 years after the initial symptoms. After necropsy, the physician described cerebral atrophy, deposits of fibrous structures in neurons in the cortical area of the brain, and extracellular plaque-like lesions (1). The features he described are currently recognized as typical of AD, whose pathology is characterized by gliosis and tissue atrophy mainly caused by the loss of synapses, especially pronounced in the cortex and hippocampus regions of the brain (2).

AD is the sixth leading cause of death in the United States and the fifth leading cause of death among the elderly population worldwide (3). Compared to the total world population, the percentage of people suffering from AD is relatively low. A census carried out in the United States in 2000 estimated that there were 4.5 million patients in the country (4), and in 2010, there were approximately 35.9 million AD patients in the world (5). However, both estimates anticipated that these numbers would increase by more than 300% by 2050. It is interesting to note that the mortality rate of AD tends to increase over the years, unlike other major causes of death, such as heart disease and cancer. The explanation for this phenomenon could be the trend toward aging of the human population, and the association of AD with this specific age group. The increases in both numbers are directly proportional (6). In 2010, it was estimated that the total cost of dementia worldwide was more than 600 billion US dollars (5). With the tendency for the size of the elderly population to increase, it is expected that costs related to AD, the most common form of dementia, will also increase. Today, owing to the small numbers of patients, little is known about AD in the general population regarding its importance to both society and the economy. However, based on existing data, it is possible that in less than 50 years AD will become a serious public health problem, with a significant socioeconomic impact.

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Molecular aspects of Alzheimer's disease

AD presents two well-characterized pathological markers, β-amyloid (Aβ) plaques and neurofibrillary tangles (Figure 1). The Aβ plaques, or extracellular senile plaques, are formed by a 42-amino acid peptide known as Aβ peptide (7,8) that is produced after cleavage of a precursor protein known as amyloid precursor protein. The released peptides form the amyloid plaques, which are insoluble, cytotoxic aggregates. These plaques are neurotoxic and result in apoptosis of neurons, local inflammation, disruption of calcium homeostasis, oxidative stress and complement activation, which are responsible for the clinical manifestations of the disease. The neurofibrillary tangles are formed by the hyperphosphorylation of the tau protein, which plays an essential role in inducing Aβ toxicity as well as mitochondrial dysfunction in AD (9-11).

Currently available treatments

Currently, there are few treatments available for AD, and they can be classified as pharmacological, psychological and immunological approaches. In the case of pharmacological treatments, there are the acetylcholinesterase inhibitors, which aim to increase the concentration of acetylcholine in the brain, covering the decrease of this neurotransmitter caused by the death of neurons (12). Another pharmacological approach is the use of glutamate receptor inhibitors such as *N*-methyl-D-aspartate receptors, whose overstimulation leads to cytotoxicity (13). The psychological treatments involve cognitive stimulation and physical exercises, such as cognitive rehabilitation, which help to deal with the limitations caused by the disease and aim to improve the patient's quality of life (14). Passive immunization with anti-Aβ monoclonal antibodies, e.g., bapineuzumab and solanezumab, has been considered an alternative AD treatment, and various monoclonal antibodies are currently being evaluated in clinical trials (15).

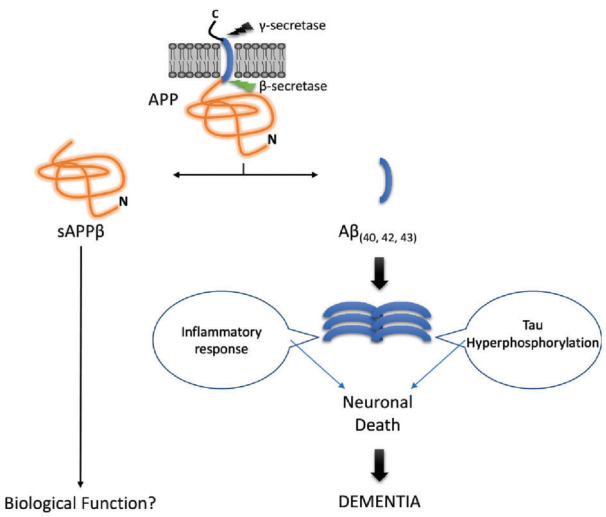

Figure 1. Alzheimer's disease (AD) in its molecular aspects. AD presents two characterized pathological markers: β-amyloid (Aβ) plaques and neurofibrillary tangles. The β-cleavage of the amyloid precursor protein (APP) results in a soluble form of this protein (sAPPβ). The β-cleavage followed by a γ-cleavage of the precursor protein results in an insoluble form of Aβ peptides, which aggregate and form plaques, causing an inflammatory response that leads to neuronal death and symptoms of dementia. The neurofibrillary tangles are formed by hyperphosphorylation of the Tau protein, which plays a role in inducing Aβ toxicity as well as mitochondrial dysfunction in AD.

Despite the availability of different treatment options, each therapeutic approach has specific limitations. The pharmacological treatments are expensive, require permanent use, and serve only to control the symptoms, not aiming to cure or reduce the progression of the disease. The psychosocial interventions improve quality of life but are not effective against the prognosis of the disease. Presently, the immunological treatments with anti-Aβ monoclonal antibodies seem to be the most promising option. However, once approved for human use, their high costs and lifelong use will pose severe limitations on the widespread use of these compounds. In addition to these disadvantages, monoclonal antibodies do not always offer the expected results. Recently, clinical trials of bapineuzumab were halted in phase 3 owing to failure to demonstrate a significant improvement in cognitive and functional activities (16).

New treatments against AD are expected to be better and cheaper than the current options by showing long-term therapeutic effects with no or reduced adverse effects. On the other hand, the possibility of preventing the disease using a vaccine approach would be preferable to the treatment of affected subjects. Accordingly, the following question can be asked: is it possible to develop a vaccine against AD that would meet these requirements?

Vaccines against Alzheimer's disease: what has been done so far?

Identifying a target for the control of the disease

Although the exact cause of AD is not yet known, there are common features observed among affected patients, the presence of Aβ plaques being one of them and arguably the most common target for immunotherapeutic approaches. Experiments carried out by Schenk and colleagues in 1999 were the first to demonstrate that immunization with the Aβ peptide could reduce the deposit of plaques in the brain of mice genetically modified to develop AD with symptoms similar to those observed in humans (17).

Noting that the generation of Aβ-specific antibodies can reduce AD symptoms, three different hypotheses were formulated to explain the effects of removal of excess Aβ from the brain (Figure 2). Firstly, the antibodies would bind directly to the peptides in the senile plaque, destabilizing the interactions of the Aβ molecule and disrupting them (18). Secondly, the Aβ-specific antibodies would bind to the plaque and promote their phagocytosis by microglial cells mediated by Fc receptors (19). Finally, the antibodies would not cross the blood-brain barrier but would bind to the circulating Aβ molecules present in the plasma of the affected subject, thereby leading to a concentration gradient that ultimately would result in the efflux of Aβ from the brain into the blood and plasma, a mechanism known as the peripheral sink model (20). Based on these facts, much of the research related to vaccines against AD has focused on the reduction of senile plaques in the brain by generating antibodies specific to the Aβ peptide through active immunization (21,22).

Vaccine approaches based on Aβ

Several vaccine approaches have been proposed with the Aβ peptide as the target antigen, employing different murine models to evaluate specific humoral responses and provide a prognosis of disease progression (Table 1). Formulations based on DNA vaccines have in common the idea of employing in-tandem fusions with immunomodulatory sequences, such as the PADRE sequence (pan human leukocyte antigen DR-binding peptide), a promiscuous nonself T-cell epitope that has been used by itself or in association with another immune modulator (23-29). These kinds of vaccine formulations have been shown to generate immune responses, evidenced by the production of antibodies specific to Aβ but without cytotoxic cellular responses.

Approaches using the $A\beta_{1-11}$ peptide derived from the fusion of Aβ with immunomodulator sequences such as PADRE, and associated with adjuvants or integrated into chimeric vaccines, such as virus-like particles, have been shown to be highly immunogenic, as seen by the induced humoral immune response with an indicative profile of T-helper 2 (Th2) cell modulation (29-34). The same applies for vaccines based on recombinant viruses, which code for epitopes of Aβ-specific to B cells, but this kind of vaccine approach remains expensive, may result in the generation of antibodies with altered epitope specificities, and carry a significant risk of inducing adverse effects (35).

Vaccine strategies using DNA or peptides against AD based on various approaches have usually induced poor immune responses (the case with DNA) or antibodies with modest avidity for the target protein (the case with peptides). In attempts to enhance the magnitude of the antibody responses, heterologous prime-boost regimens have been tested, in which the first priming dose is followed by a boost based on a different delivery approach that promotes the expansion and selection of B cells with a high degree of avidity for the target antigen (36,37). The immune responses achieved with such a vaccine regimen were particularly promising, especially that proposed by Lambracht-Washington and colleagues (37) based on a prime-boost immunization strategy in which a shortened Aβ peptide ($A\beta_{1-42}$) containing both B- and T-cell epitopes was used. The volunteers were initially primed with a synthetic peptide, and then boosted with a DNA vaccine

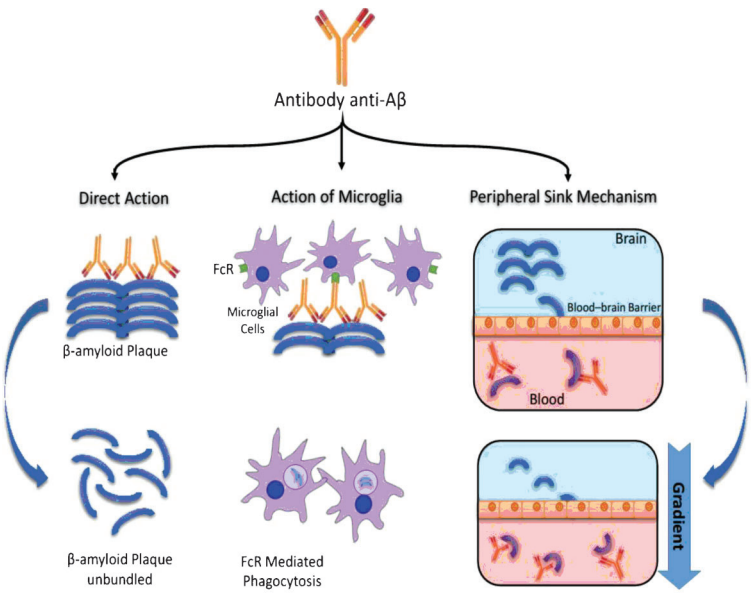

Figure 2. Mechanism of β-amyloid (Aβ) removal via Aβ-specific antibodies. There are three hypotheses for the mechanism of action of anti-Aβ. The first involves the direct action of the antibody against the Aβ plaques, where the binding of the antibody destabilizes the plaques. The second involves the action of microglia, which leads to the phagocytosis of Aβ mediated by Fc-receptors (FcR). Lastly, there is the peripheral sink mechanism hypothesis, in which the antibody binds to and removes Aβ present in the plasma, generating a net efflux of Aβ from the brain to the plasma.

Table 1. Examples of vaccine formulations currently being tested against Alzheimer's disease in animal models.

Vaccine formulation	Animal model tested	Reference
DNA vaccines		
pCMVE/MDC-3Aβ11-PADRE-C3d	C57BL/6 mice	Movsesyan et al. (23)
pVAX-3Aβ11-PADRE-Thep	Rhesus macaques	Evans et al. (24)
p(Aβ3-10)10-C3d-p28.3 + bupivacaine	B6C3-Tg mice	Guo et al. (25)
pVAX-6Aβ15-T-Hc/Hc-C	$PDAPP^{V717I}$ mice	Yu et al. (26)
pN-3Aβ11-PADRE-Thep + electroporation	Rabbits	Ghochikyan et al. (27)
pCMVE/MDC-3Aβ11-PADRE + LT-IS	3xTg-AD mice	Davtyan et al. (29)
gL-Abx4-Fc-IL-4	B6C3-Tg mice, rabbits and cynomolgus monkeys	Matsumoto et al. (28)
Epitope/protein/VLP-based vaccines		
$2Aβ_{1-11}$-PADRE-MAP + QuilA	APP Tg 2576 mice	Petrushina et al. (30)
Adeno-10 × Aβ3-10 + CpG	B6C3-Tg mice	Li et al. (31)
2Aβ1-6-VLPQβ	APP24 mice	Wiessner et al. (32)
pDisplay-Aβ	APP23 mice	Bach et al. (33)
2Aβ1-11-PADRE-MAP + LT-IS	3xTg-AD mice	Davtyan et al. (29)
Prime-boost approach		
Aβ1-42 peptide prime/Aβ1-42 DNA + QuilA	B6SJLF1/J mice	Lambracht-Washington et al. (37)
AdenoPEDI-(Aβ1-6)1 prime/pCA-PEDI-(Aβ1-6)	C57BL/6J mice	Kim et al. (36)

encoding the same target antigen. What is interesting in this approach is that although T cells were present in the initial stage of vaccination, the T cell level later decreased, indicating that the boost with the DNA vaccine promoted the activation of T_{reg} cells, which were responsible for the low reactivity of Aβ-specific T cells (37).

Another vaccine approach against AD has been based on the other pathological marker of the disease as the target antigen, the neurofibrillary tangles produced by hyperphosphorylation of the Tau protein (11). This approach is not without challenges: there has been at least one report of neuroinflammation in mice as a result of repeated immunization with phosphorylated Tau-derived peptides, raising concerns about the safety of this kind of vaccine (38).

Clinical studies: past, present, and future

The first clinical trial involving a vaccine against AD was carried out in 2000 with the aggregated human $Aβ_{1-42}$ peptide combined with a saponin-based adjuvant (AN1792) (39). The results of this phase I trial provided evidence of the safety and tolerability of the vaccine based on a multiple-dose regimen. However, adverse inflammatory effects, leading to subacute meningoencephalitis, were observed in nearly 6% of the volunteers enrolled in a phase II trial with the AN1792 vaccine, which ended dramatically after the death of one patient despite improvements in the clinical symptoms and reduction of senile plaques in several other patients (40-43). Subsequent studies showed that the adverse effects observed in the AN1792 trial could be ascribed to the toxicity mediated by activated T cells reacting with self-antigens, resulting in an inflammatory autoimmune response (40,42). The $Aβ_{1-42}$ T-cell epitopes were located in the central region and carboxyl-terminus of that Aβ peptide (44).

In order to avoid undesirable inflammatory effects, the amino-terminus of the Aβ peptide, in which the B-cell epitopes were located, was subsequently used as an antigen target for anti-AD vaccines (45,46). CAD106, a vaccine candidate composed of a B-cell epitope ($Aβ_{1-6}$) is currently being tested in a clinical trial. In this vaccine, the peptide was genetically fused to the bacteriophage Qβ coat protein to generate virus-like particles, each containing 180 copies of the coat protein of the phage. Phase I trials were performed to evaluate the safety, tolerability, and immunogenicity of this vaccine. The absence of adverse effects, e.g., autoimmune inflammation, allowed the start of phase 2 trials (47). Another example that will soon have clinical trials initiated is the Lu AF20513 vaccine, composed of three B-cell epitopes ($Aβ_{1-12}$) fused to two Th-cell epitopes derived from the tetanus toxoid, P2 and P30 (34). The major purpose of this vaccination is the activation of memory Th cells, which are preexistent in the general segment of the population that has been vaccinated with a conventional vaccine against tetanus, facilitating the quick response against Aβ even in the elderly population (34).

Conclusion: is a vaccine capable of preventing or treating AD feasible?

Studies performed during the last century allowed the identification of distinct features of AD, such as the

accumulation of Aβ plaques in the brain, and the relationship of these deposits with the clinical manifestations. These observations opened perspectives for new therapeutic interventions for the control of the disease, particularly during the last decade. Studies focused on vaccines have advanced significantly and now represent a promising therapeutic alternative for disease control, based on the generation of antibodies against the Aβ peptide. These advances were accompanied by retreats, as in the case of the first clinical trials, that provided important lessons for researchers, who have deepened their knowledge and developed alternatives for the design of safer and more effective vaccines for the control of AD. Recently, the possibility of targeting proteins other than Aβ has been tested, and promising results are expected to be seen with the Tau protein, but clinical data are still lacking, and should be pursued in this kind of approach.

There has been some debate as to whether targeting Aβ would be sufficient for an immunologically based therapy, because the role of senile plaques in the clinical picture appears to be just the tip of the iceberg. Indeed, the fact that promising results generated in animal models have not been reproduced in clinical trials suggests that Aβ alone might not be the only target antigen for active immunization. The finding that the Tau protein could play a role as a target antigen for the control of AD adds further expectations regarding new vaccine formulations with better performance in human beings.

The multifactorial nature of the AD pathology makes it difficult to propose a ''perfect target'' for the development of drugs or immunotherapy. In the face of these difficulties, the contribution of passive immunotherapy based on monoclonal antibodies might find a more promising role in the treatment of AD. Nonetheless, recent results based on active immunization suggest that, in addition to a direct therapeutic effect in subjects already affected by the onset of disease, immunization should also be considered as a conventional prophylactic approach. Testing vaccines that are able to induce specific antibodies prior to the manifestation of symptoms may be an alternative to prevent amyloid being deposited in the senile plaques, according to the peripheral sink hypothesis. So far, such a preventive approach has not been experimentally proven but deserves future effort and support.

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