Novel Immunotherapeutic Procedures for Prevention of Alzheimer’s Disease
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Introduction
There are two main modalities of immunotherapy for Alzheimer’s disease (AD) under investigation, based on previous results with animal models and clinical trials. The first approach was passive immunotherapy, with the administration of monoclonal Aβ-specific antibodies, although some disadvantages and side-effects were described in the literature [1]. A second approach is active immunization with the Aβ42 antigen-tested mouse models [2]. Based on this methodology, posterior studies have tried to avoid the adverse activation of T-cell-mediated immune response that probably caused negative effects in patients. Although there are different immune compounds being given to APPswe/PS1dE9 double-transgenic mice to achieve the same goal, one particular study has taken a new approach by delivering Aβ42 in a novel immunogen-adjunct manner consisting of Sphingosine-1-Phosphate (S1P)-containing liposomes, administered to APP/PS1 transgenic mice before and after the detection of AD-like pathology in the brain [3]. The results from this novel vaccine (EB101) indicate that active immunization significantly prevents and reverses the progression of AD-like pathology and also clears prototypal neuropathological hallmarks in those transgenic mice studied. This new approach seemed to strongly induce T-cell, B-cell and microglial immune response activation, avoiding the Th1 inflammatory reaction. These beneficial effects were achieved by using a physiological adjuvant formed by naturally-occurring phospholipids, which have already been proven safe and efficient in several other types of vaccines, such as influenza. In the EB101 vaccine, the S1P-phospholipid plays a key role in the anti-inflammatory reaction, probably by acting as a neuronal regenerating agent in in vitro and in vivo studies [4].

Rationale for immunotherapy in AD
There are consistent reasons why immunotherapy should work in AD [5], based on studies published during the past decade. Some of these reasons are: (a) Aβ-amyloid plaques and their aggregated, protofibrillar and oligomeric precursors contain immunologic neo-epitopes that are absent from the full-length Amyloid Precursor Protein (APP), as well as from its soluble proteolytic derivatives restricted to the brain tissue; therefore, β-amyloid-based immunotherapies designed to selectively target pathologic neo-epitopes present on Aβ oligomers, protofibrils or fibrils, should not cause autoimmune disease in unaffected tissues throughout the organism. (b) Aβ-amyloid buildup precedes neurodegeneration and functional loss, and the prevention of its formation or its removal can be expected to result in the slowing or the prevention of neurodegeneration. (c) Aβ-amyloid can cause the formation of neurofibrillary tangles in vitro and in vivo, as has been supported by animal models and tissue culture. These findings strongly suggest that the removal of β-amyloid bears the potential to correct not only β-amyloid-related toxicity but also to prevent the formation of neurofibrillary tangles. (d) Conformational changes of endogenously occurring proteins and the formation of insoluble aggregates are commonly associated with neurodegeneration and brain disease, so the removal or prevention of these pathologic protein aggregates is also a therapeutic goal in the principle of immunotherapy. (e) Immunotherapy works in experimental animals and in initial clinical trials: both active immunization and passive antibody transfer consistently reduce brain β-amyloid load, improve β-amyloid-related memory impairments, and protect neurons against degeneration in many independent experiments using different mouse models and primates.

Mechanisms of action in immunotherapy
Some hypotheses [6] concerning the basis of immunotherapeutic mechanisms are currently under investigational scrutiny, such as: (a) Plaque breakdown: A specific antibody is capable of entering the brain and opsonizing Aβ burden plaques, and during the immune response process the plaques are fragmented and phagocytized by microglial cells. (b) Peripheral sink: This is based on the hypothesis that antigen-antibody complex formation at the brain periphery isolates β-amyloid from the damaged brain areas and avoids the deposition of new Aβ plaques. The increased levels of Aβ in serum also raised another possibility, in which the Aβ is sequestered from the brain directly into the bloodstream by changing the Aβ blood-brain balance rate to enhance clearance of soluble Aβ. (c) Aggregation inhibitor: Aβ represents a key molecular target in AD intervention therapy, and agents that can efficiently prevent its aggregation and accumulation or potentiate its clearance might represent remarkable therapeutic benefits. Among the main potential inhibitors are those related to the β- and α-secretase enzymes which are required in the abnormal processing of Aβ [7].

To confront and prove these hypotheses, different strategies involving Aβ immunotherapy are currently under investigation by the scientific community, such as: (a) Direct Aβ42 immunization in transgenic mouse models has already provided clinical feedback of Aβ immunotherapy [8]. This direct immunization approach aims to stimulate the immune system response, activating the T-cells, B-cells and microglia in the brain. (b) Another strategy of active immunization involves the administration of Aβ conjugated synthetic fragments...
bound to a carrier protein, thus avoiding potential problems associated with mounting a T-cell response directly against Aβ [9]. (c) The third type of immunotherapeutic strategy involves the passive administration of monoclonal antibodies, developed outside the patient’s body, directed against Aβ [10]. This method does not stimulate the immune system to play an active role in fighting the infection as do the previous two active methods.

**Tau-based Immunotherapy**

Recent studies have observed increased levels of tau oligomers in the early stage of AD, previous to detection of Neurofibrillary Tangles (NFT) formed by aggregation and accumulation of the microtubule-associated protein tau [11]. Since Aβ immunotherapy presents a limited clearance effect of tau aggregates in dystrophic neurites, the development of an alternative therapy that directly targets pathological tau has become crucial. In the last decade, several approaches have been taken to treat AD by targeting tau, such as (a) the inhibition of tau hyperphosphorylation, by using a kinase inhibitor of soluble aggregated tau formation, and also to prevent related motor deficits [12], although unwanted side-effects might be found due to its important physiological role; (b) Activation of the proteolytic pathway, by the degrading action of calpain [13] and puromycin-sensitive aminopeptidase [14]; (c) Stabilization of microtubules, treating tauopathies by functionally binding and stabilizing microtubule with MT-binding protein tau [15] and paclitaxel, a drug proven effective in restoring the affected axonal transport and motor impairments [16]; and (d) Tau clearance by immunotherapy. In this case, the tau active vaccination uses phosphorylated antigens of tau fragments associated with neurofibrillary tangles [17], that results in an efficient reduction of both soluble and insoluble tau active fragments, reducing phosphorylated NFTs in the AD-like mouse brains.

**Pre-clinical findings**

Pre-clinical studies have provided clear evidence that Aβ immunization therapy provides protection and reverses the pathological effects of AD in transgenic mouse models [18]. This strategy seems to improve cognition performance [19] after Aβ1-40 immunization, in addition to causing an effective reduction in Aβ pathology. A recent immunization study has proven that a fragment of the Aβ peptide bound to polylysines activated the immune response that results in the diminishing of AD-like pathology in APP transgenic mice. This report reinforces the notion that the immune-conjugate approach is an effective means of Aβ immunotherapy and also that the entire Aβ peptide is not necessary for its efficacy, and is in accordance with the hypothesis that specific antibodies directed against the amino-terminal and/or central region of the amyloid peptide provide beneficial protection against amyloid pathology. Passive immunization studies have also been conducted with promising experimental results showing that a humoral response alone, without Aβ cellular response, is sufficient to reduce the β-amyloid burden and to reverse memory deficits [20].

**Current perspectives**

Among the drugs and vaccines currently under development to treat the pathological effects of AD, bapineuzumab, solanezumab, CAD106 and EB101 are the most promising ones. Solanezumab is a monoclonal antibody raised against Aβ13-28, recognizing an epitope in the core of the amyloid peptide, binding selectively to soluble Aβ and with low affinity for the fibrillar Aβ form [21], presenting fewer adverse events than bapineuzumab, which binds to Aβ amyloid plaques more strongly than soluble Aβ [22]. There are a few other monoclonal antibodies against Aβ that present properties different from those of bapineuzumab, such as (a) PR-04360356, which specifically targets the free carboxy-terminus of Aβ1-40; (b) MABTS102A, which binds with equally high affinity to Aβ monomers, oligomers and fibrils; (c) GSK933776A, which targets the N-terminus of Aβ. Specific anti-Aβ antibodies are present in pooled preparations of intravenous immunoglobulin (IVig or IGIV), which has already been approved by the FDA for the treatment of a variety of other neurological aspects. Current results from these studies have shown that IVig treatment may also be an efficacious alternative approach in the treatment of AD neuropathologies [23].

Avoiding both the strong Th1 effects of QS-21 adjuvant and the T-cell epitopes at the C-terminus of Aβ, CAD106 consists of a short N-terminal fragment of Aβ attached to a virus-like particle, with no additional adjuvant [24]. This therapeutic agent is currently in phase II trials. Affiris is testing two short 6-amino-peptides (AD01, AD02), administered with aluminium hydroxide as adjuvant, that mimic the free N-terminus of Aβ and therefore cause cross-reactivity with the native peptide in phase I trials [25]. In terms of prevention and therapeutic treatment approach, the EB101 vaccine showed for the first time the effectiveness of combining a liposomal immunogen-adjuvant with an Aβ antigen to induce an effective immunological response combined with an anti-inflammatory effect in preclinical studies using APP/PS1 transgenic mice [3]. The EB101 vaccine immunization process has shown a marked positive effect as a preventive and therapeutic treatment, reducing amyloidosis-induced inflammation as an effective Th2 immunomodulator. Moreover, this vaccine proved to stimulate innate immunity and enable effective phagocytosis to clear amyloid and neurofibrillary tangles, among the major hallmarks of AD-like neuropathology observed. Phase I clinical trials are expected to reinforce these consistent results. However, a few other vaccines are currently under development, and recent studies have opened new perspectives in the immunization approach to AD pathology, in particular, the gene-gun-mediated genetic immunization with Aβ9-28 gene [26], showing that self-tolerance can be broken in order to produce a humoral response to the Aβ1-40 peptide with minimal cellular response.

**Conclusion**

The current experimental studies to develop anti-amyloid treatments using immunological approaches are expected to provide a framework based on the amyloid hypothesis in a near future. This approach will be tested in transgenic AD animal models and it is expected to be an effective therapy method to prevent the AD neuropathological effects. Active immunization strategies are being planned and developed in order to maximize endogenous antibody production as well as to minimize T lymphocyte reactions. However, there is still a void in the identification and validation of AD biomarkers for the diagnosis of early pathological effects in the brains of AD patients. Together with the evaluation of the therapeutic efficacy of immunotherapies currently under development, are major hurdles to be addressed in the near future by researchers and investigators worldwide. In conclusion, based on studies reported over the past two decades, there is a strong and solid evidence to suggest that Aβ immunotherapy may have disease-modifying potential leading to an effective therapy in AD both in animal models and in AD patients. However, alternative emerging
neuroprotective approaches such as neurotrophic factor enhancers, neurotrophins, and stem-cell treatments are also under investigation.

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