Invited Editorial

Combating Detrimental Aging-related Uprisings: Impacts of Immunotherapy and Vaccination

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Abstract

Of central nervous system (CNS) disorders, Alzheimer’s disease (AD) continues to grow among elderlies causing a big challenge for the health systems worldwide. While no conventional therapies can cure the AD-mediated CNS disorders such as dementia, passive and active vaccination of this disease seem to be a promising preventive treatment modality. Technically, the immunotherapy of AD is based on either active immunization through injection of amyloid beta42 (Abeta42) as antigen, or passive immunization by injection of anti-Abeta42 monoclonal antibodies (mAbs). So far, some peptides (e.g. CAD106, ACC001 and Affitope) and mAbs (e.g. solanezumab, gantenerumab, and crenezumab) have even been taken into phase 2/3 clinical trials. Despite promising clinical outcomes from these trials, it seems that we are yet to engineer effective immunomedicines to fight the aging-related CNS disorders (e.g. AD-mediated dementia). Here, in this mini-review, we discuss the hallmarks of AD and highlight the essentiality of this imperative challenge.

Keywords: Alzheimer’s disease, Aging, CNS disorders, Immunotherapy, Vaccination

Introduction

Conceptually, immunization/vaccination is one of the most effective approaches in combating most infectious and/or contagious diseases as well as any other diseases that are manifested by over-expression of undesired biomarkers. Recently, numerous studies have been conducted worldwide to fight life-threatening diseases such as malignancies and age-related CNS disorders using personalized immunomedicines. This dominion still needs some advancement towards production and application of effective personalized immunotherapies targeting array of biomarkers involved in development of the CNS-mediated disabling diseases such as AD-mediated dementia. Of note, AD is a disabling CNS disorder that creates a lot of pressure on the health care systems in modern societies where life expectancy is high and so is the aging-mediated diseases. As the most common basis of dementia among elderlies, AD is known as one of the major contributors to disability and dependency worldwide. The immunization/vaccination of AD needs a better understanding of molecular pathology of the disease which may promote development of new personalized vaccination and cell therapy modalities.

Although the initiation, development and progression of AD are not fully comprehended, the basis of AD pathogenesis is deemed to be due to the accumulation of abnormally folded amyloid-beta protein (Aβ) and/or hyperphosphorylated tau protein (ptau) in the brain. It is worthy of noting that the AD is most common cause of dementia that contributes in approximately 60-70% of the cases, and is considered as one of the major priorities of public health worldwide. Furthermore it results in devastating disabilities contributing to morbidity among elderlies. AD requires substantial health-care interventions. Unfortunately, the number of patients with dementia is estimated to be roughly doubled every 20 years (i.e., 66 million in 2030 and 115 million in 2050) [1]. Further, the cell-based vaccination and immunotherapy modalities granted great hopes. It is deemed that these initiatives will change the face of therapy towards personalized medicines for most of the human diseases such as age-related disorders. Nowadays, the reprogramming of human cells is no longer a fiction and in reality we are able to isolate the desired cells from any individuals, remodel the molecular features and inject them back. Despite being in its infancy era, this approach seems to be the only rational treatment of some diseases even though we need to devise much more effective modalities based on a holistic panel of intricate phenomena specific for each individual.

Since in human aging a complex network of biomolecules are involved, preventive vaccination and immunotherapy may bestow a cost-effective and efficacious means to overcome these hurdles.

To tackle this issue, various preventive, alleviative and treatment modalities have been conducted, including: medications for memory loss, behavioral and sleep changes. All these therapies are aimed to target several biomolecules that are believed to be involved in development of AD. This review provides some insights on the hallmarks of AD and also discusses inevitable challenges in fighting the AD-mediated dementia.

Hallmarks of Alzheimer’s disease

Among several biomarkers involved in initiation/development of AD, amyloid beta (Abeta) and tau proteins play central roles and are briefly discussed in relation with inflammation.

Amyloid beta

In the mid-1980s, after discovery of Abeta, formation of plaques through aggregation of Abeta peptides was considered as an important hallmark of Alzheimer’s brain abnormality [2]. Abeta is the chief component of plaques that, together with neurofibrillary tangles (NFTs) because of tau protein aggregation, causes the age-related neurodegenerative disorders in particular AD. Taken all investigations into consideration, the parent amyloid precursor protein (APP), which is a transmembrane protein consisting of 695–770 amino acids, is deemed to be sequentially cleaved by a group of enzymes complexes – known as α-, β- and γ-secretases. APP enzymatic cleavage non-amyloidogenic (i.e., by α and γ-secretases at a position 83 amino acids from the carboxy terminus) and amyloidogenic
(i.e., by β- and γ-secretases) at a position 99 amino acids from the carboxy terminus) pathways [3]. While the non-amyloidogenic pathway precludes the formation of Abeta, the amyloidogenic pathway leads to the generation of intact Abeta peptides as approximately 90% of 40 residues in length (Aβ40) and about 10% of 42 residues in length (Aβ42). The latter peptide seems to be more hydrophobic entity and hence more prone to fibril formation than Aβ40, resulting in formation of cerebral plaques. Several enzymes are involved in an intricate activities of α-, β- and γ-secretases. The α-secretase functionality is based on the enzymatic activity of A Disintegrin And Metalloprotease (ADAM) family (i.e., ADAM9, ADAM10 and ADAM17) and possesses metalloprotease, integrin-binding, intracellular signaling and cell adhesion activities [4-6]. The β-secretase enzymatic activity is mediated by APP-cleaving enzyme 1 (BACE1) that is a type I integral membrane protein of the pepsin family of aspartyl proteases [7,8]. And finally, the γ-secretase was reported to be a complex of enzymes encompassing presenilin 1 or 2 (PS1, PS2), anterior pharynx defective, nicastrin and presenilin enhancer 2.

**Tau protein**

Tau protein is responsible for development of neurofibrillary tangles (NFTs) that occur intraneuronally. It was discovered in mid-1970s, since then a large number of researchers showed its impact in initiation and progression of AD. In the late 1980s, NFTs were shown as the aggregates of the tau proteins, abnormally hyperphosphorylated forming tangles in the brain parenchyma. Tau protein belongs to the microtubule-associate proteins (MAPs) and its encoding gene locates on the long arm of chromosome 17 (i.e., at 17q21) encompassing 16 exons. This protein can stabilize the microtubules which seems to occur as coordinated actions of kinases and phosphatases, in which the phosphorylation of tau protein modulates its interaction with microtubules impacting the axonal transportation of vesicles along the microtubules mediated by kinesin protein [9]. Conformationally, tau protein shows six different isoforms whose sizes vary from 352 to 441 amino acid residues. In AD, it has been shown that this protein fails to maintain the cytoskeleton of the axonal bodies well-organized in large part due to altered microtubules-binding capacity (perhaps because of conformational changes and/or misfoldings) [10,11]. For instance, in the neurons affected in AD, tau has been reported to lose the binding capacity to the tubulin while failing to promote the assembly of microtubule, and hence, precluding the organization of microtubule within the affected neurons [12,13]. Such inhibitory impacts on vesicular trafficking within the neuronal cells appears to affect the exocytosis functions and mitochondrial dissemination. Pathologically, the abnormal tau seems to trigger the initiation aggregation of tau in AD patients. Further, proteolytic cleavage of the tau protein is of note phenomena which seems to be an alternative mechanism for the occurrence of the abnormal aggregation of tau [14]. All these aberrant events may lead to some inevitable neuronal loss within the areas of the brain that are responsible for the cognitive functions. Also, it should be noted that aberrant alteration in tau protein structure (e.g., abnormal phosphorylation, acetylation, glycation, nitration, truncation) are deemed to play a pivotal role in development of AD; readers are directed to see [10,15-17].

**Inflammation**

Inflammation has also been reported to be a central player in Alzheimer’s brain abnormality. In fact, development of Abeta-based plaques and ptau-mediated tangels seems to sustain chronic activation of primed microglia, activating some important inflammatory functions and hence producing inflammatory bio-agents such as cytokines and chemokines by the affected cells. Such aberrant phenomena are deemed to impair the microglia and also impact the neighboring cells such as astrocytes, oligodendrocytes and neurons, which can result in neurodegeneration and neuronal loss. In neurodegenerative diseases, the core contribution of the innate immune system occurs via resident microglia and perivascular macrophages, while other blood-derived myeloid cells (e.g., dendritic cells and monocytes) impose little influence upon the initiation/progression neurodegeneration phenomena [18]. Having highlighted all these, the inflammatory impacts in AD are yet to be fully understood; readers are directed to see [18-20].

**Insulin and insulin receptor impacts**

In AD – apart from less than 5% of all cases due to mutations of three genes that result in the permanent development of disease – all other cases are sporadic in origin related to aging. AD is often associated with some anomalous engagements with the neuronal insulin signaling pathway, in which Abeta seems to bind to the insulin receptor competitively in the early-onset familial AD while this receptor can be desensitized in the late-onset sporadic AD by noradrenaline and/or cortisol [21]. In addition to the glucose metabolism, there exist compelling evidence that insulin and insulin receptor modulate the metabolism of APP, the intracellular formation and the release of APPs and Abeta into the extracellular space, and also phosphorylation of tau protein [21-24]. Taken all, we must fully comprehend the role of insulin in the brain and address issues such as how brain cells get their energy needs. For example, the glucose insufficiency in neuronal system could be moderately compensated through the use of endogenous brain substrates (i.e., amino acids and fatty acids). Accordingly, in the early-onset familial AD, glutamate is metabolized to retain the ATP level at a normal state, while the use of glutamate as an energy source may inadvertently result in production of neurotoxic ammonia in the brain and thus interfere with normal function of mitochondria [21]. All these findings suggest that we need to implement new strategies to stave off AD, for which personalized immunotherapy seems to bestow much more effective means.

**Blood-brain barrier (BBB) impacts in AD development**

Principally, in healthy condition, the BBB control the traverse of endogenous and exogenous compounds into the brain and hence maintains the homeostasis of the central dogma. In the presence of astrocytes and pericytes, the brain microvasculature endothelial cells form a very tight barrier setting with great integrity by creating tight junctions consisting of proteins such as zonula occludens; readers are directed to see [25-30]. However, in AD, calcium influx is induced by Aβ resulting in perturbed intracellular Ca2+ homeostasis and hence loss of BBB integrity [31,32].

**Immunization of Alzheimer’s disease**

Generally, it is of essential demands to control the dreadful AD ideally by active or passive immunization. Further, it should be highlighted that the stem cells isolated and transplanted into the intact brain of rodents were shown to be able to pursue their right trajectory in the host parenchyma resulting in (a) differentiation into functional neural lineages, (b) targeted relocation towards the damaged regions of the brain, and (c) proliferation and maturation towards functional neural cells. The intravenously administered neural precursor cells are also able to migrate into the damaged areas of brain and induce functional recovery [33-36]. These findings have raised several clinical trials to attain the proof-of-concept in human subjects (e.g., ClinicalTrials.gov Identifier: NCT01547689). While the stem cell therapy of AD has just been begun to combat the dementia, a large number of studies have been conducted to examine the potentials and benefits of the immunotherapy and vaccination modalities (Table 1).

To the best of our knowledge, the immunotherapy of AD has been capitalized on (a) active immunization against amyloid β42 (Abeta42), in which patients receive injections of monoclonal antibodies (mAb) against Abeta42. Of the examined active or passive immunizations approaches, some of the peptide-based vaccines used for active immunizations were CAD106, ACC001 and Affiltope that have been undergone phase 2/3 clinical trials. In addition, some monoclonal antibodies (mAbs) such as solanezumab, gantenerumab, and crenezumab have been investigated in phase 2 and 3 clinical trials [1], which have resulted in some limited clinical benefits (Table 2).

Altogether, it appears that there is an immediate need for an initiative advocating the advancement of investigation on the immunization of AD. While the currently used medicines for AD simply alleviate the symptoms of AD-mediated dementia, recently studies on active/passive immunotherapy appear to be treatment strategies.

Further, targeted therapy of AD has been shown to successfully reduce the accumulation of Abeta, resulting in profound prevention of downstream
pathology of the disease. Both immunotherapies using antibodies against AN-1792 peptide and ptau protein indicate their clinical benefits even though such impacts seem to be more effective in early stages of amyloid accumulation. Despite promising clinical impacts of the active vaccination with Abeta peptide, clinical trials using this strategy were halted in part because of the development of meningoencephalitis in number of patients treated with such modality. Although the active vaccine therapy and passive immunization using humanized anti-Abeta mAbs are judged to be effective on the basis of clinical and pathological analyses in some patients, their overall clinical outcomes are still under question. This review highlights some benefits and challenges of the active vaccination and passive immunotherapy against AD. Passive immunization through administration of few humanized anti-Abeta mAbs has been taken into clinical trials even though these immunotherapies have still some issues to be solved. Of note, DNA vaccines have been developed to combat AD. This approach seems to be a simple strategy that can be easily modified and administered with no need for any types of adjuvants. Several research groups have capitalized

Table 1. Selected clinical trials conducted for immunotherapy and vaccination of Alzheimer’s disease.

<table>
<thead>
<tr>
<th>Study</th>
<th>Identifier</th>
<th>Biological</th>
<th>Phase</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study to Evaluate Safety, Tolerability and Immunogenicity of Vaccine (UB 311) in Subjects With Alzheimer’s Disease</td>
<td>NCT00965588</td>
<td>UB 311</td>
<td>1</td>
<td>Completed (Aug 2011)</td>
</tr>
<tr>
<td>18-months Safety Follow-up Study of AADvac1, an Active Tau Vaccine for Alzheimer’s Disease (FUNDAMANT)</td>
<td>NCT02031198</td>
<td>AADvac1</td>
<td>1</td>
<td>Currently recruiting</td>
</tr>
<tr>
<td>Study Evaluating Safety, Tolerability, And Immunogenicity Of ACC-001 In Subjects With Mild To Moderate Alzheimer’s Disease</td>
<td>NCT00479557</td>
<td>ACC-001 and QS-21</td>
<td>2</td>
<td>Completed (Feb 2013)</td>
</tr>
<tr>
<td>Phase Ib Follow-up Study to Evaluate Long-term Safety and Tolerability of Immunization With AFFITOPE AD01 Applied During AFFIRIS 001</td>
<td>NCT00711139</td>
<td>AFFITOPE AD01 and AFFIRIS 001</td>
<td>1</td>
<td>Completed (Dec 2009)</td>
</tr>
<tr>
<td>To Investigate the Safety and Tolerability of Repeated Subcutaneous Injections of CAD106 in Alzheimer’s Patients</td>
<td>NCT01023685</td>
<td>CAD106</td>
<td>2</td>
<td>Completed (June 2013)</td>
</tr>
<tr>
<td>AAB-001 in Patients With Mild to Moderate Alzheimer’s Disease</td>
<td>NCT00112073</td>
<td>Bapineuzumab</td>
<td>2</td>
<td>Completed (March 2012)</td>
</tr>
<tr>
<td>Safety Study of Passive Immunization for Patients With Mild to Moderate Alzheimer’s Disease</td>
<td>NCT00174525</td>
<td>AAB-001</td>
<td>2</td>
<td>Unknown</td>
</tr>
<tr>
<td>Effect of LY2062430 on the Progression of Alzheimer’s Disease (EXPEDITION 2)</td>
<td>NCT00904683</td>
<td>LY2062430</td>
<td>3</td>
<td>Completed (Dec 2012)</td>
</tr>
<tr>
<td>Progress of Mild Alzheimer’s Disease in Participants on Solanezumab Versus Placebo (EXPEDITION 3)</td>
<td>NCT01900665</td>
<td>Solanezumab</td>
<td>3</td>
<td>Currently recruiting</td>
</tr>
</tbody>
</table>

Note: Data were obtained from https://clinicaltrials.gov.

Table 2. Biologics used for passive or active immunization of AD.

<table>
<thead>
<tr>
<th>Biologic</th>
<th>Description</th>
<th>Immunity</th>
<th>Manufacturer</th>
<th>Clinical impacts</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAD106</td>
<td>Multiple copies of Aβ1-6 peptide derived from the N-terminal B cell epitope of Aβ, coupled to a Qβ virus-like particle</td>
<td>Active</td>
<td>Novartis Pharmaceuticals Corp. (Basel, Switzerland)</td>
<td>Discontinued after phase 2 trials</td>
</tr>
<tr>
<td>ACC001</td>
<td>A conjugate of multiple short Aβ fragments linked to a carrier made of inactivated diphtheria toxin</td>
<td>Active</td>
<td>Janssen Pharmaceuticals, Inc. (Titusville, NJ, USA)</td>
<td>Successful completed clinical phase 1 studies. Under phase 2 studies increased production of anti-Aβ Ab, decreased TH1 response (successful phase 1), Under phase 2 studies</td>
</tr>
<tr>
<td>Affitope (AD01)</td>
<td>Short peptides, mimicking parts of the native beta amyloid (Aβ) sequence as the antigenic component</td>
<td>Active</td>
<td>AFFIRIS (Vienna, Austria)</td>
<td></td>
</tr>
<tr>
<td>Affitope (AD02)</td>
<td>A synthetic peptide of six amino acids that mimics the N-terminus of Aβ, that lacks the most common T cell epitope (amino acids 15-42 of Aβ) but including the B cell epitope (amino acids 11-15 of Aβ) , it may allow for the production of anti-Aβ antibodies while minimizing a pro-inflammatory TH1 response</td>
<td>Active</td>
<td>AFFIRIS (Vienna, Austria)</td>
<td></td>
</tr>
<tr>
<td>AN-1792</td>
<td>Synthetic full-length Aβ peptide with QS-21 adjuvant</td>
<td>Active</td>
<td>Janssen Pharmaceuticals, Inc. (Titusville, NJ, USA); Pfizer Inc. (New York City, NY, USA)</td>
<td>Discontinued after phase 2 trials</td>
</tr>
<tr>
<td>Solanezumab</td>
<td>A humanized monoclonal whole antibody</td>
<td>Passive</td>
<td>Eli Lilly (Indianapolis, IN, USA)</td>
<td>Failed in phase 3 trials, limited benefits</td>
</tr>
<tr>
<td>Gantenerumab</td>
<td>An investigational fully human mAb against Aβ</td>
<td>Passive</td>
<td>Roche (Basel, Switzerland)</td>
<td>Failed in phase 3 trials, limited benefits</td>
</tr>
<tr>
<td>Crenezumab</td>
<td>A humanized whole mAb targeting human 1-40 and 1-42 Aβ</td>
<td>Passive</td>
<td>AC Immune (Lausanne, Switzerland); Genentech, Inc. (San Francisco, CA, USA); Pfizer Inc. (New York City, NY, USA); Johnson &amp; Johnson (New Brunswick, NJ, USA)</td>
<td>Failed in phase 3 trials, limited benefits</td>
</tr>
<tr>
<td>Bapineuzumab</td>
<td>A humanized monoclonal whole antibody against Aβ</td>
<td>Passive</td>
<td></td>
<td>Failed in phase 3 trials, limited benefits</td>
</tr>
</tbody>
</table>
on DNA vaccines based on Abeta targeting, upon which significant reduction has been shown for the induced Abeta in AD model mice without side effects. It should be noted that AD as a neurodegenerative disorder is under intensive investigation towards development of effective immunotherapy modalities. Further, there exist a significant correlation between pathology of AD and accumulation of extracellular Abeta or intracellular ptau fibrils which can be therapeutic targets for intrabody immunotherapy; readers are directed to see [37]. Paganiotti and co-workers reported on targeting of the intercellular pathway of Abeta production through an intrabody, sFvβ1, which was directed to the EFRI epitope, a tetrapeptide next to the β-secretase cleavage site of β-amyloid precursor protein. While sFvβ1 binds to newly synthesized APPs in endoplasmic reticulum and escorts it during secretory transport to membrane, it protects APP from the pathologic β-secretase-mediated cleavage, a process producing toxic Abeta [38]. In addition, a large panel of anti-tau intrabodies were produced to target the microtubule-associated protein tau, involved in AD. However, in vivo studies and therapeutic efficacy of all above intrabodies remains to be determined.

As a long-standing goal of AD research, our today’s challenges and even tomorrow’s confrontations seem to be the personalized prevention of diseases through reprogramming of the immune system. The strategy can be applied against life-affecting and age-related diseases such as malignancies [39] and inflammatory disorders. However, for the development of such modalities, we will need to advance our knowledge upon the mechanism(s) of these diseases in a holistic manner. To be precise, targeting a single marker of fatal diseases no longer can be the case, but rather a cascade of key molecular markers need to be targeted by the endogenous defense machineries of patient, even far before the onset of the disease through implementation of the cell-mediated immunotherapy and vaccination as novel personalized holistic therapy modality.

References
31. Kook S.Y, Seok Hong H, Moon M, Mook-Jung I: Disruption of


