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The Central Role of T-Cell memory in Alzheimer's disease vaccination

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Abstract

Alzheimer's disease (AD) is the most common progressive neurodegenerative brain disease as well as the most common dementia among the elderly. In the future as the average lifespan continues to extend, the number of AD patients will continue to grow. Amyloid-beta (Aβ) peptides, in both soluble oligomeric, and insoluble forms, are key in the neuropathogenesis of AD and have thus been a therapeutic target for vaccines. Multiple Aβ vaccination strategies in animal models of AD have demonstrated a marked reduction in both amyloid burden and neurocognitive deficits. Due to the success of these studies, initial human clinical trials of an active Aβ vaccine were conducted. These were discontinued due to the development of meningoencephalitis in approximately 6% of the vaccinated AD patients. Studies examining the brains of Aβ-vaccinated patients developing meningoencephalitis implicate Aβ-reactive T-cell subsets as major components of this deleterious response to active Aβ vaccination. To subvert possible meningoencephalitis resulting from Aβ vaccination a second generation of vaccines has been more recently developed. These however have met with little success in humans. To build on these findings, an understanding of the role of T-cells in vaccination against Aβ is presented in this review. Various methods of Aβ immunotherapy are reviewed including studies in both animal models and humans. Recent works suggest that Aβ-derived peptides delivered intranasally or transcutaneously results in effective clearance of

Aβ plaques and improvement of cognitive function in animal models of AD. Moreover, undesired T-cell reactivity appeared to be considerably reduced compared with other active immunization strategies. In spite of the past clinical studies, these findings imply that Aβ vaccination may be both efficacious and safe depending route of delivery, adjuvant choice, and Aβ epitope administered.

Introduction

First characterized in 1907, Alzheimer's Disease (AD), is the most common cause of dementia and affects 5 million people in the United States. The prevalence of the disease increases exponentially with advancing age.[1] About 1% of persons aged 65 years have AD, and the percentage increases to almost 50% in persons greater than 85 years of age. In the United States, this phenomenon is in part secondary to life expectancy increasing by some 50%, from 50 years of age in 1900, to 75 years of age in 2000. This has resulted in a modern threefold increase in the percentage of persons over 65. The problem is worldwide however. Thus a prophylactic or curative treatment for AD would have enormous benefits.

The cerebral amyloid beta (Aβ) protein deposits in AD are primarily found postmortem in two brain regions: scattered in the extracellular neocortex and limbic system and in the walls of cerebral blood vessels.[2] These deposits consist of amyloid fibrils in a β-pleated sheet conformation made up of mixed polymers of the 40 and 42 amino acid Aβ peptides.[1,3] The DNA sequence coding for Aβ is a small portion of a larger gene encoding a transmembrane amyloid precursor protein (APP). APP undergoes proteolysis by two enzyme complexes, beta (β) and gamma (γ) secretases, to generate the Aβ peptide.[4-6] Additionally, increasing evidence supports soluble oligomeric forms of Aβ, particularly dimers as an equally, if not more, neurotoxic species.[7,8]

The biology of T-cell memory in vaccination

An understanding of immunological memory and its relationship to an effective vaccine against AD is a key issue in medical neuroimmunology. Primary immunization or "priming" causes antigen-specific T-cells to proliferate, yielding large pools of effector T-cells which move into peripheral tissues to combat pathogens or proteins. A portion of these "primed" T-cells develop into memory cells, which provide immediate protection and the ability to mount a faster and effective secondary immune response.[9,10]

Memory T-cells comprise at least two subsets, each with distinctive migratory and effector capacities.[11-13] Cells of the first subset are somewhat similar to the effector cells generated in the primary response in that they lack lymph node-homing receptors (L-selectin and chemokine receptor type 7 [CCR7]) and express receptors for migration into inflamed tissue. Upon re-encounter with antigen, these *effector memory T-cells* (TEM) quickly release interferon-gamma (IFN-γ), Interleukin (IL)-4, or perforin. T-cells in the second subset express L-selectin and CCR7 as do naïve T-cells) and do not possess immediate effector function. These *central memory T-cells* (TCM) have a low activation threshold and, upon restimulation in secondary lymphoid organs, proliferate and differentiate into effectors.[12-15]

Many of these T-cells are positive for CD8 (cluster of differentiation 8), a transmembrane glycoprotein that serves as a co-receptor for the T-cell receptor (TCR). Importantly, the distribution of peripheral T-cell subsets in young and healthy elderly individuals is distinctly different, marked by decreased naïve cells and increased clonal expansions of memory CD8+ major histocompatibility complex (MHC) class I-restricted T-cells.[16,17]

As with the CD8 T-cells the differentiation process for CD 4 (cluster of differtiation 4) T-cells is controlled by both TCR and cytokine stimulation.[18] CD4+ T-cells have traditionally been divided into two subsets based on their cytokine expressing repertoire. That is, T-helper 1 (Th1) cells are pro-inflammatory while T-helper 2 (Th2) cells are considered anti-inflammatory.[19] In regard to Th1 cells, prolonged TCR stimulation in the presence of IL

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(interleukin) -12 or IL-4 promotes terminal differentiation to effector Th1 or Th2 cells respectively. Notably, AD is marked by primarily Th1 response in the brain.[20,21] A short TCR stimulation period and TGF-β preserve the cells in a central memory-like stage.[14,18,22]

Thus in both CD8+ and CD4+ T-cells, terminal differentiation is not a necessary consequence of T-cell activation. The generation of *resting* intermediates that endure as central memory cells provides the immune system with a reserve of highly sensitive cells that can be quickly recruited in secondary immune activation responses; thus generating large pools of potent CD4 or CD8 effector cells.[23,24] This is important since peripheral T-cell activation likely leads to CNS immune activation when the blood-brain barrier (BBB) permeability may be compromised, as in AD.[25]

Aβ-reactive T-cells

Recently Monsonego and colleagues, demonstrated T-cell reactivity to the more immunogenic peptide Aβ$_{1-42}$[26] instead of Aβ1-40.[7] On the other hand, T-cells were infrequently stimulated by the N-terminal region 1-28 residues. An analysis of Aβ T-cell epitopes and their restriction to HLA (human leukocyte antigen)-DR class II showed Aβ is processed and presented through MHC by APCs and that Aβ-specific T-cell proliferation is mediated through MHC-TCR interactions. Thus, Aβ is able to confer an adaptive immune response in the periphery.[27]

There have been at least two other previous studies in humans measuring Aβ$_{1-40}$ reactive T-cells in the peripheral circulation. The first[26] reported Aβ-induced T-cell proliferation in young and elderly controls but that these T-cells were not present in AD patients. It was hypothesized Aβ-reactive peripheral T-cells were anergized in AD patients. On the other hand, others found activation and expansion of Aβ-reactive T-cells in the elderly and patients with AD indicates Aβ is captured by local APCs in the brain, and that these APCs migrate to secondary lymph nodes; inducing T-cell activation.[7] Although Aβ deposition occurs in elderly humans that do not have overt signs of AD, there appears to be increased T-cell reactivity to Aβ in patients with AD, since in contrast to elderly subjects, all patients in Monsonego and colleagues' study with AD had some Aβ reactivity.[7] Such reactivity could reflect an endogenous reaction to Aβ deposition which we observed as local innate immune response in AD brain *post-mortem*.[27]

Thus it could be said that some Aβ reactive T-cell pools in the CNS (i.e. those present in AD patients)[26,27] enhance the cognitive decline process whereas as those present in the periphery in individuals of any age[25] seem neuroprotective.

Animal Aβ immunization

Evidence directly linking Aβ to symptoms of AD first came from transfecting a mutant human APP (amyloid precursor protein) gene from a patient with hereditary AD into the murine genome (APP-transgenic mouse); yielding cerebral Aβ plaques and cognitive deficits.[4] Several transgenic mouse models of AD that express human, mutant APP genes, alone or in combination with human, mutated presenilin and tau genes now exist.[28]

Regarding vaccination strategies, Schenk and colleagues[29] first showed vaccination with Aβ$_{1-42}$ and Freund's adjuvant ameliorated β-amyloid generation in brains of young transgenic mice and decreased β-amyloid in aged mice with pre-existing AD pathology including quantity and density of Aβ plaques in the brain, with related improvements in neuritic dystrophy and gliosis.[29] Later, active vaccinations in transgenic mice, nonhuman primates, and other species further confirmed these results.[30-34]

Passive transfer of anti- Aβ antibodies is also able to efficiently reduce β-amyloid pathology in animal.[34-41] The vaccine-mediated clearance of β-amyloid pathology in animal models is reflected by the recovery of neuronal and cytoskeletal morphology,[42-44] by improvement of neurotransmission,[31,44,45] and[45,46] and most importantly by improved cognitive functions.[47-49]

From animal studies, two theories (not mutually exclusive) of the mechanisms by which Aβ antibodies work have been developed. First, Fc-mediated uptake and clearance of Aβ antibody complexes by microglia has been demonstrated.[50] Second, evidence of a net efflux of Aβ peptide out of the brain and into the serum and the cerebrospinal fluid (CSF), as a result of its binding and mobilization by Aβ antibodies, has been obtained.[51,52]

In addition to these paradigms, because primarily local innate inflammation occurs in AD brain, an immune *balance* by induction of specific adaptive, Th2, immune responses has been demonstrated to be beneficial in animal models of AD.

Some examples of this can be seen in the literature whether investigators are exploring immunization with forms of Aβ peptide, or by immunization with a gene encoding Aβ. Hong-Duck and colleagues demonstrated an adenovirus encoding 11 tandem repeats of Aβ$_{1-6}$ fused to the receptor-binding domain (Ia) of Pseudomonas exotoxin A (PEDI) or AdPEDI-(Aβ$_{1-6}$)$_{11}$ can induce anti-inflammatory Th2 immune response in mice. They then went on to explore whether a DNA prime-adenovirus boost regimen could elicit a more robust Th2 response using AdPEDI-(Aβ$_{1-6}$)$_{11}$ and a DNA plasmid encoding the same antigen. All mice administered DNA prime-adenovirus boost regimen were positive for anti-Aβ antibody,

while, out of seven mice immunized with only AdPEDI-(Aβ$_{1-6}$)$_{11}$, four mice developed anti-Aβ antibody. The mean anti-Aβ titer induced by the DNA prime-adenovirus boost regimen was some7-fold greater versus the AdPEDI-(Aβ$_{1-6}$)$_{11}$ alone.[53]

Further, genetic immunization with the *Aβ$_{1-42}$* gene in AD transgenic mice effectively elicited a humoral immune response without a significant T-cell-mediated immune response to the Aβ peptide.[54]

Additionally, papillomavirus-like particles (VLP) have also been employed which display Aβ$_{1-9}$ protein repetitively on the capsid surface. This peptide contains a functional B cell epitope, but lacks T-cell epitopes. Rabbit and mouse vaccinations were well tolerated and induced high-titer antibody against Aβ, that effectively inhibited assembly of Aβ$_{1-42}$ peptides into neurotoxic fibrils *in vitro*.[55] In PSAPP mice trends for reduced brain Aβ deposits, and increased Aβ in plasma, suggested efflux from the brain to periphery as well.[51,52] These results are important because the Th2 response is salutary in the CNS in AD.

Also to induce Th2-polarized immune responses, some groups used other B cell epitopes of Aβ[53] such as Aβ$_{1-15}$, Th2-type adjuvants such as IL-4,[17,56,58] Alum,[56,57] mannan,[56] mono - phosphoryl lipid A, cholera toxin B subunit, *E. coli* enterotoxin,[59] and transcutaneous[60] or mucosal vaccination.[33] Thus overall it seems immunization modalities conferring predominantly Th2 type immune responses are safer for AD prevention and treatment.[7, 52,61-63]

Development of anti- Aβ vaccination in humans

An obstacle to introducing a vaccine mediated immune response in humans is that AD patients already experience a chronic inflammatory process surrounding neuritic plaques. In AD an innate immune response is triggered by local production of Aβ protein.[27] Innate immune involvement is evident from the complement proteins of the by activation of microglia, resulting in the release of pro-inflammatory cytokines and chemokines (for further review see,[21]). Additionally, Aβ fibrils can be modified by endogenous sugars to form "advanced glycation endproducts" (AGEs), which in turn activate pro-inflammatory signal transduction pathways in which the receptor for AGEs (RAGE), and oxygen free radicals (as second messengers) are produced in excess. Aβ and AGEs activate transcription factors leading to upregulation of neurotoxic cytokines including IL-1, IL-6 and TNF-α.[64-67] The inflammatory pathology (microgliosis, astrocytosis, complement activation, increased cytokine expression and acute phase protein response) is thought to be a secondary response to early accumulation of brain Aβ.

As in animal models, it is possible that an

adaptive immune response accompanied by Th2 type cytokine predominance may indicates a "successful" response to vaccination. This would be observed as a lack of meningoencephalitis and antibodies bound to neuritic plaques facilitating antibody mediated clearance of Aβ from the brain. In this case, the innate Aβ brain inflammation should halt after the plaque burden has decreased, and this should be accompanied by a stabilization, or even recovery of cognitive function. Indeed, accumulation of Aβ in the brain has been suggested to be caused by an impaired capacity to clear the protein in AD patients.[2]

Initial phase I and phase II clinical (Elan/Wyeth AN1792) trials were conducted with aggregated Aβ$_{1-42}$ and the QS-21; a Th1 response-activating adjuvant.[68,69] In the late stages of the phase I trial polysorbate 80, an emulsifying agent, was added to the active vaccine. It was at this time that immune responses shifted from a predominantly Th2-polarized response to a pro-inflammatory Th1 response[69] marked by meningoencephalitis and vascular T-lymphocyte infiltrations.[70] The mechanism of this self- reaction is unknown. However the appearance of the inflammation prior to detection of Aβ antibodies in some of the patients suggests a T-cell-mediated immune reaction to Aβ, which caused bystander damage to the brain, was at work. In the later phase II trial in 2002 by Elan and Wyeth, 18 further patients (6% of the patients treated with the active vaccine) developed subacute aseptic meningoencephalitis after having received mostly two doses, and in some cases one or three of the initially planned six doses of the active vaccine.[71] Two participants suffered ischemic strokes as well. The active vaccination also led to a humoral immune response in some of the vaccinated patients with significantly increased IgG and IgM titers.[68,72,73] These antibody titers were unrelated to the occurrence or severity of meningoencephalitis.[71] Further, one individual with severe meningoencephalitis had no detectable antibody titers suggesting the humoral response was not required to cause meningoencephalitis.

Long-term follow-up of the Elan phase I/II study cohort of actively vaccinated patients revealed increased titers of antibodies reacting with brain β-amyloid plaques.[74] Indeed 19 patients with serum β-amyloid plaque-reactive antibodies showed slower cognitive decline over a 1-year period than did nine patients who did not develop these antibodies. Although the cohort size was quite small, this finding suggests anti-β-amyloid antibodies as protective.[75] However, when all participants were analyzed, no overall significant differences on cognitive performance were found between the placebo and treatment groups.[72,76] This may have been related to the relatively small decline in ADAS-cog (Alzheimer's Disease Assessment Scale-cognitive subscale) scores in the placebo group.[72,77]

Autopsy tissues from patients who died from unrelated causes showed patchy patterns of β-amyloid plaque clearance associated with increased antibody titers, with some regions almost totally free of β-amyloid plaques[70,71,78,79] In several cases, β-amyloid plaque reductions were associated with increased brain tissue concentrations of water and detergent-soluble forms of Aβ,[80] suggesting some biological plaque-clearing activity of antibodies occurred as a result of vaccination. No β-amyloid clearance was observed in a single case without detectable antibody titers.[80] These results point to biologically important effects of Aβ antibodies on β-amyloid plaque pathology in AD.

However with increasing evidence supporting oligomeric Aβ as the more neurotoxic species,[7,8] there may be a low therapeutic value for clearing of already well established parenchymal plaques for the improvement of cognitive decline in human AD patients. Prevention or efficient clearing of toxic oligomeric Aβ may be more effective. Because of Aβ reactivity in AD patients,[7] the use of a full-length Aβ peptide, and the use of a Th1 adjuvant (QS21), it is comprehensible how the Elan vaccination may have caused aseptic meningoencephalitis. Further, it is important to point out that another potential problem with the Elan trial is that the vaccine consisted solely of Aβ. Activation of B cells requires T-cell help and because the vaccine consisted solely of Aβ, the T-cell help required for the induction of strong IgG antibody responses was, by necessity, directed against Aβ itself.

To reconcile this side effect of the human study with all the previous animal trials not demonstrating it, it is necessary to understand that increased T-cell Aβ reactivity has not been demonstrated in APP transgenic mice. This may be secondary to their high levels of peripheral Aβ and resulting induction of T-cell tolerance.[7] It has also been suggested the encephalitis may also result from antigen spreading and expansion of T-cell clones specific to myelin antigens such as myelin basic protein.

These combined data from the pre-clinical experiments and the initial clinical active vaccination trials led to the next generation of AD vaccines, presumably safer active vaccines with less strong Th1-cell activating formulations and with C-terminally truncated Aβ fragments (since the Aβ C-terminus contains T-cell activating epitopes). One advantage of the second generation conjugate vaccines being tested is that T helper epitopes are either absent or provided by a conjugate, not Aβ.[81] Several active vaccination approaches are currently tested in clinical trials (*www.clinicaltrials.gov*) at the time of this review including the Merck V950 trial, the Novartis/Cytos CAD-106 trial using a VLP-linked N-terminal Aβ peptide fragment, as well as the Affiris Affitope AD01 and AD02 active vaccination trials with Aβ peptide mimetics. The Elan/Wyeth ACC-001 phase II active vaccination trial with an N-terminal Aβ peptide fragment conjugated to a carrier protein was suspended due to transient skin lesions in one patient in the study.

Efforts are underway to determine the basis for the adverse inflammatory reaction seen in the first generation of AD vaccines, and to model it in animals. In this regard, we developed a transcutaneous (t.c.) active Aβ vaccination in a transgenic mouse model of AD. PSAPP mice showed high Aβ antibody titers. Most importantly, t.c. immunization with Aβ$_{1-42}$ plus a cholera toxin (CT) adjuvant resulted in significant decreases in cerebral Aβ$_{1-40,42}$ levels coincident with increased circulating levels of Aβ$_{1-40,42}$ suggesting brain-to-blood efflux of the peptide. Importantly there was no brain T-cell infiltration or cerebral microhemorrhage.[60] Other groups have also found no abnormal effects in APP transgenic mouse models to which Aβ antibodies have been administered, and such mice have shown robust behavioral improvements and clearing of brain Aβ deposits.[34,82] It is important to consider however, that the antibody response in mice is elicited against the human form of Aβ, which has an amino acid sequence that differs from the mouse form. Thus, production of high titer antibodies against the non-functional human Aβ are observed[60,83] while limiting the humoral and cell-mediated response against the endogenous mouse Aβ sequence. Therefore, the autoimmune response in mice is less likely to confer collateral damage by targeting functionally important APP and soluble Aβ.[84] It should also be noted that a number of other groups have shown that passive vaccination increases the degree of cerebral amyloid angiopathy and associated microbleeds.[38,41,85,86] Salloway and colleagues (2009) explored vaccination of Bapineuzumab, a humanized anti- Aβ monoclonal antibody, as a potential passive vaccine strategy in a multiple ascending dose, safety, and efficacy study in mild to moderate AD (N=234). Patients received 6 antibody infusions, 13 weeks apart, with final assessments (via ADAS-Cog) at week 78. Primary efficacy outcomes in this phase 2 trial were not significant and six vasogenic edema patients experienced transient symptoms.[87]

Th2 memory effector cells are a requirement for antibody production but in the elderly, the predominant T-cell population is Th1 cells, which generate the proinflammatory cytokines when stimulated by Aβ vaccination. We and others hypothesize this predisposes individuals to develop AD as well as other age related diseases.[88-91] Indeed in the elderly the Aβ-specific T-cell clones are composed largely of CD8-positive cytotoxic T-cells, which can lyse cells

presenting the Aβ sequence.[92] Thus any increase in T-cell mediated neuroinflammation from vaccination runs the risk of accelerating neuronal loss in AD patients who are already have a low threshold for CNS damage due to the high levels of oxidative stress and inflammation.[93,94] A further complication relates to a difficulty in being able to limit the inflammatory response to the insoluble Aβ in plaques while sparing soluble Aβ monomers, the parent protein [amyloid precursor protein (APP)] and neurotrophic APP fragments[84] such as s-APPα.[95]

In further relation to the possible toxicity of vaccinations, the majority of the Aβ epitope is localized to the extracellular portion of APP. As such, antibodies to Aβ might recognize native cell surface APP, leading to complement activation, subsequent opsonisation, and brain cell injury or death. Importantly, self-reactive T-cells of low-to-moderate binding affinity are not all automatically deleted during negative selection in the thymus.[96,97] Indeed a portion of autoreactive T-cells undergo positive selection and maintain the normal immune repertoire.[98] Moreover, although the CNS is known as *immunologically privileged*, activated T-cells can routinely penetrate the BBB.[99-101]

Conversely, Th2 cytokine promoting or producing cells may have salutary regulatory properties. Aβ administered intranasally to APP transgenic mice induced anti-Aβ antibodies and partial clearance of Aβ plaques. This was in conjunction with infiltration into the CNS of small numbers of mononuclear cells expressing anti-inflammatory Th2 cytokines IL-4, IL-10, and TGF-β.[33] Interestingly, nearly all human Aβ-reactive T-cell lines showed a Th2 phenotype. Thus it is possible mucosal immunization could boost this lineage, enhancing clearance of Aβ by both stimulating Aβ antibody production and modulating microglial activation at sites of Aβ plaques, with a minimal risk of harmful T-cell responses in the CNS. Further, overexpression of Th-2 cytokine TGF-β in the CNS of APP transgenic mice resulted in a significant reduction of Aβ plaques via promotion of microglial clearance of the peptide.[102]

Taken together, both human and animal findings imply that Aβ vaccination may be both efficacious and safe provided the route of delivery, adjuvant choice, and Aβ epitope choice are properly combined to avoid deleterious T-cell activation in the CNS.

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