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AD Vaccines: Conclusions and Future Directions

Thomas Wisniewski

New York University School of Medicine, Millhauser Laboratory, HN 419, 550 First Avenue New York, NY 10016, USA, thomas.wisniewski@nyumc.org

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia worldwide, affecting approximately 37 million people currently. By 2050, according to some estimates, 1:85 persons worldwide will be affected by AD [1]. Currently available treatments for AD provide largely symptomatic relief with only minor effects on the course of the disease. There is an urgent need for better therapeutic interventions. Besides immunomodulation, numerous other approaches are being studied, which include anti-amyloid- β ($A\beta$) aggregation agents, secretase inhibitors/modulators blocking $A\beta$ production, tau aggregation blockers, and various neuroprotective strategies [2]. Perhaps the greatest hope for an intervention that shall significantly impact disease progression in the near future comes from the vaccination approaches. Certainly in AD transgenic (Tg) mouse models $A\beta$ directed immunization has been spectacularly successful using a wide variety of methods. However, as outlined in the prior chapters, significant unanswered questions remain for the current and future human trials as to what is the best design of a vaccine, what is the best target and when should therapy start?

THE PAST ACTIVE IMMUNIZATION HUMAN EXPERIENCE

As discussed in several of the preceding chapters, the striking biological effect of $A\beta$ directed vaccination in mouse models, without apparent side effects, led to the launch of clinical trials with a vaccine designated as AN1792 which contained pre-aggregated $A\beta$ 1-42 and QS21 as an adjuvant. This type of vaccine design was aimed to induce a strong cell mediated immune response, since QS21 is known to be a strong inducer of Th-1 lymphocytes [3]. The initial trial was conducted in the UK and involved 80 patients with mild to moderate AD [4]. This trial was designed to assess the antigenicity and the toxicity of multiple dose immunization with the full length $A\beta$ 1-42 peptide with the QS21. 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 biased response to a proinflammatory Th1 response [5]. 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 prematurely terminated when 6% of vaccinated patients manifested symptoms of acute meningoencephalitis (18 out of 298 subjects) [3, 6, 7]. Autopsies performed on a limited number of trial patients suggested that striking $A\beta$ clearance of parenchymal plaques had occurred, similar to what had been reported in the animal studies, confirming the validity of this approach for amyloid clearance in humans [7-12]. Several significant problems were apparent on review of the pathological and clinical data from these trials. Some of the pathologically examined cases showed a deleterious T-cell reaction surrounding cerebral vessels, suggestive of an excessive Th-1 immune response. It appeared that the immune reaction triggered by AN1792 was a double-edge sword, where the benefits of a humoral response against $A\beta$ were overshadowed in some individuals by a detrimental Th1 mediated inflammatory response [7, 13]. Additional striking features were the persistence of amyloid in cerebral vessels, as well as unaltered tau

immunoreactive neurofibrillary tangles (NFTs) and neuropil threads in regions of cerebral cortex where plaque clearing had apparently occurred, compared to regions without clearing [10-12]. Hence, this initial vaccination approach did not address vascular amyloid or NFT related pathology. Furthermore, despite the apparent success in amyloid clearance, the clinical cognitive benefits were very modest when the active vaccination group was compared to the placebo group [14, 15]. However, there are a number of possible ways by which each of these shortcomings can be addressed, as discussed in the preceding chapters.

SUMMARY OF PROBLEMS TO RESOLVE FOR DEVELOPING ENHANCED AD VACCINATION

1) When to Start Vaccination?

A critical issue is when is the optimal time to begin immunomodulation? As discussed by Tarawneh and Holtzman, by the time patients have begun even the mildest stages of AD, the neuropathological changes are already well established with high numbers of both diffuse and neuritic plaques distributed widely [16]. The importance of starting early is supported by data from the follow up study of 80 patients in the phase I AN1782 trial, of whom 8 had an autopsy [15]. This study showed that despite evidence of very significant amyloid plaque removal in 6 out of the 8 autopsy subjects, which correlated with the anti-A β titer, in the overall group there was no evidence of improved survival or an improvement in the time to severe dementia [15]. It is hoped that if immunization is begun early, A β -lowering may well be effective in preventing NFT formation, which can occur as a down-stream effect of A β -related toxicity [17, 18], and thus be associated with more robust cognitive benefits. This idea is supported by data in transgenic mice in which A β antibodies reduced both beta-amyloid and early, but not late forms of hyperphosphorylated tau [19]. Therefore, A β immunotherapy could be active in preventing neurofibrillary tangle formation without affecting numbers or the morphology of preexisting neurofibrillary tangles. Hence there is a need for identification of markers predicting the conversion from normal aging to very mild dementia/mild cognitive impairment. These include cerebral spinal fluid biomarkers such as increased p-tau/A β 42 ratios in cognitively normal individuals which enhance the probability of conversion to MCI (mild cognitive impairment) [20]. Early FDG-PET changes in hippocampal glucose metabolism can predict the conversion of normal cognition to pathologically verified AD [21]. Studies in AD Tg models suggest that paramagnetic amyloid binding ligands utilizing magnetic resonance imaging have potential for early amyloid detection and following treatment effects [22, 23]. However currently, direct imaging of amyloid depositions with agents such as PIB, using PET imaging, is the most promising method for identification of early amyloid deposits and identifying patients who will likely convert to early AD [24,25].

2) Better Pre-Clinical Models of AD

Better models for pre-clinical testing of vaccination approaches need to be identified and/or developed. As discussed in preceding chapters, there are many shortcomings to current Tg models of AD pathology. Relatively few of the vaccination approaches being developed have been tested in non-human primates which may provide more accurate models of the type of immune responses that might be elicited in aged humans [26-28]. As discussed by Vasilevko and Head, aged beagles represent another natural model of A β pathogenesis. It is striking that in a 22 month active immunization trial in this model, despite ~80% reductions in cortical A β immunoreactivity, little cognitive improvement on multiple measures of learning and memory could be detected [29]. However, a significant improvement in executive function was found, mirroring the improvements seen only in the z-score of the Neurological Test Battery among patients in the AN1782 trial. These results reinforce the

need to begin immunomodulation very early in the disease progression, focusing on preventing A β deposition rather than clearance of preexisting lesions.

3) Improvements in the Design of Vaccines

As discussed in the chapters by Town and Ghochikyan, there is a need to redesign the next generation of vaccines to avoid the excessive Th1 stimulation that resulted in encephalitis in a proportion of the AN1792 patients. Concurrently the formulation of any active vaccine also has to overcome the problem of immunosenescence in the target patient population. As discussed, one promising approach taken by several investigators is to alter the sequence of the A β peptide immunogen in order to remove or alter the major Th1 stimulator sites in the carboxyl terminus and the middle portion of A β , while focusing on the major Th2 stimulator site in the amino terminus [30-34]. These A β homologous peptide immunogens can be combined with various co-stimulator epitopes. One promising approach is combination with a synthetic, non-natural Pan HLA DR-binding epitope PADRE [34] or linkage to viral-like particles [35-37] to induce a primarily humoral immune response. These can be further combined with other immunostimulator carriers. For example the A β Th2 amino terminal epitope can be combined with PADRE and macrophage derived chemokine (MDC) in a DNA epitope vaccine to drive robust Th2 responses [38].

The choice of adjuvant is also an important consideration. The use of polysorbate 80, a strong Th1 stimulate adjuvant, in the AN1792 trial is one likely contributing factor to the encephalitis in a minority of patients. Use of adjuvants such as alum, which drive primarily a Th2 response, is preferable [29, 39]. The route of immunization also plays an important role. Stimulating mucosal immunity by vaccinating nasally, *via* the gut or transcutaneously has been shown to drive strong Th2 responses [40-42]. An alternative, non-mutually exclusive approach to enhance vaccine design is to stimulate innate immunity and enable microglia/macrophages to phagocytose amyloid deposits. Over 20 yrs ago, H. Wisniewski noted that while brain-resident macrophages were unable to phagocytose amyloid, brain-infiltrating macrophages are plaque competent [43]. A number of recent studies suggest that only a small percentage of plaques are associated with peripheral origin macrophages and that these are required for plaque clearance [44-46]. Vaccination approaches based on this knowledge are now being developed. Stimulation of peripheral macrophages to enter the CNS and phagocytose amyloid has been achieved by using CpG oligodeoxynucleotides to activate Toll-like receptor 9 [47], *via* blockade of the CD40/CD40L interaction [48] and by blockade of TGF β -Smad2/3 innate signaling pathway [49]. These innate immunity stimulatory approaches can be used alone or in combination with adaptive immunity stimulation.

4) Understanding the Mechanism(s) of Vaccination

As outlined in the chapters by Golde, Das and Levites and by Morgan, there are multiple non-mutually exclusive mechanisms by which active or passive immunization may work. These possible mechanisms include: 1) catalytic dissolution of amyloid deposits by antibodies; 2) Fc mediated macrophage phagocytosis of amyloid; 3) non-Fc mediate macrophage amyloid clearance; 4) a peripheral sink whereby A β is drawn out of the brain into the peripheral circulation; 5) antibody mediated neutralization of A β oligomer toxicity and 6) facilitated transport of IgG-A β complexes out of the brain *via* a Fc receptor. It is likely that vaccination is effective *via* several of these pathways concurrently; however, gaining a greater understanding of which is the most important mechanism in patients is critical for future vaccine development.

5) What is the Most Appropriate Target for Vaccination?

Abundant evidence both *in vivo* and *in vitro* suggests that the most toxic species of A β are oligomers or A β derived diffusible ligands (ADDLs) [50, 51]. As discussed in several of the chapters, but more specifically by Lambert, Velasco, Viola and Klein, active vaccination or use of monoclonal antibodies that specifically target oligomers would be an ideal way to block A β related toxicity. A small number of preclinical studies suggest that this methodology is potentially powerful and in need of further development [52-54]. An additional important factor is that emerging evidence suggests that A β peptides have normal physiological functions in the brain, such as modulating LTP (long-term potentiation) [55]. Targeting oligomeric A β structures would avoid the potential of interfering with these physiological functions.

6) How to Address Vascular Amyloid?

A significant problem with vaccination approaches, as discussed by Wilcock and Colton, is clearance of vascular amyloid and the associated hemorrhage risk. Vascular amyloid or congophilic angiopathy (CAA) is present in virtually all AD cases, with approximately 20% of AD patients having “severe” CAA [56]. Furthermore CAA is present in about 33% of cognitively normal elderly, control populations [57]. So far the majority of active and passive immunization studies, as well as the human autopsy data from the AN1792 trial, have suggested that vascular amyloid is ineffectively cleared, with the limited clearance being associated with microhemorrhages. There are a number of ways this may be avoided. It is of interest that studies in aged beagles, which were immunized over a longer period and relatively early in the disease progression, had a tendency for CAA reduction [29]. Hence, it is possible that this complication can be avoided if immunization is started very early in the course of disease (prior to significant CAA deposition). Alternatively, stimulation of innate immunity appears (at least in initial studies) to be an effective means of reducing vascular amyloid (~80% reduction) without associated microhemorrhages [47]. It may also be that vaccine mediated disruption of parenchymal amyloid may need to be coupled to methods of increasing A β peptide egress across the blood-brain barrier (BBB). As discussed by Kokjohn and Roher, parenchymal amyloid vaccine mediated clearance is associated with sharp increases in the amounts of water soluble A β 40/42 in the brain parenchyma, which can subsequently be deposited in vessels [58]. Hence the therapies discussed by Deane, Bell, Sagare and Zlokovic that increase LRP1 expression or reduce RAGE activity in the BBB may need to be concurrently given with vaccination to avoid this complication.

7) How to Target Tau Related Pathology?

Autopsy data from the AN1782 trial indicated that despite marked reductions in amyloid burden, tau related pathology was relatively unaffected. Tau related pathology could be addressed in several ways: 1) It is hoped that if immunization is begun early, A β -lowering may well be effective in preventing NFT formation, as discussed above. 2) Development of active and passive immunization approaches which target the common abnormal high β -sheet conformation of A β and hyperphosphorylated tau oligomers could be effective against both pathologies. It is possible that stimulation of innate immunity may be effective against tau related pathology as well as amyloid pathology. 3) Anti-A β immunization may need to be combined with tau based immunomodulation. Initial data suggests that immunotherapy directed to tau pathological conformers may reduce abnormally phosphorylated tau levels in association with behavioral benefits [59].

CONCLUSION

Abnormal protein conformation is thought to be not only the underlying pathogenesis of AD but also of a long list of neurodegenerative conditions, such as prion disease, Parkinson's

disease and Huntington's chorea. Immunomodulation holds great promise as a disease altering therapeutic approach for all these disorders. For example, it has been shown that prion directed mucosal vaccination can prevent infection from an exogenous source [60, 61]. Ultimately, the successful application of immunomodulation approaches may lead to dramatic novel interventions for a wide spectrum of age associated diseases.

ABBREVIATIONS

ADDLs	A β derived diffusible ligands
AD	Alzheimer's disease
Aβ	Amyloid- β
BBB	Blood-brain barrier
CAA	Congophilic angiopathy
NFT	Neurofibrillary tangle
Tg	Transgenic

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