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Antibody Responses, Amyloid- β Peptide Remnants and Clinical Effects of AN-1792 Immunization in Patients with AD in an Interrupted Trial

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Abstract

Post mortem examinations of AN-1792-vaccinated humans revealed this therapy produced focal senile plaque disruption. Despite the dispersal of substantial plaque material, vaccination did not constitute even a partial eradication of brain amyloid as water soluble amyloid- β (A β) 40/42 increased in the gray matter compared to sporadic Alzheimer's disease (AD) patients and total brain A β levels were not decreased. Significant aspects of AD pathology were unaffected by vaccination with both vascular amyloid and hyperphosphorylated *tau* deposits appeared refractory to this therapy. In addition, vaccination resulted in the consequential and drastic expansion of the white matter (WM) amyloid pool to levels without precedent in sporadic AD patients.

Although vaccination disrupted amyloid plaques, this therapy did not enhance long-term cognitive function or necessarily halt neurodegeneration. The intricate involvement of vascular pathology in AD evolution and the firm recalcitrance of vessel-associated amyloid to antibody-mediated disruption suggest that immunization therapies might be more effective if administered on a prophylactic basis before vascular impairment and well ahead of any clinically evident cognitive decline. Amyloid- β is viewed as pathological based on the postmortem correlation of senile plaques with an AD diagnosis. It remains uncertain which of the various forms of this peptide is the most toxic and whether A β or senile plaques themselves serve any desirable or protective functions. The long-term cognitive effects of chronic immunotherapy producing a steadily accumulating and effectively permanent pool of disrupted A β peptides within the human brain are unknown. In addition, the side effects of such therapy provided on a chronic basis could extend far beyond the brain. Eagerly seeking new therapies, critical knowledge gaps should prompt us to take a more wholistic perspective viewing A β and the amyloid cascade as aspects of complex and many-faceted physiological processes that sometimes end in AD dementia.

An Alzheimer's disease (AD) diagnosis leaves patients with few treatment options and no hope of a cure. In light of this grim reality, the demonstration of senile plaque disruption through amyloid- β (A β) immunotherapy has been both stunning success and irresistible force. Despite the emergence of an unanticipated adverse event during the first human clinical trials [1], numerous new immuno-therapeutic approaches are undergoing evaluation. Although a clearly promising approach, several critical issues regarding the ultimate benefits and consequences of immune disruption of senile plaques remain unresolved.

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It is widely accepted that in AD, senile plaques provoke deleterious responses and induce injury to surrounding neurons, so their prevention or removal would avert damage to surrounding tissue. Despite the fact that senile plaques are associated with pathological conditions, these deposits may actually represent the safest means to minimize the threats from excess production of potentially toxic soluble A β peptides [2-4]. An unanticipated consequence of liberating A β from plaques may be to grant these molecules easier access to compartments from which they had been safely excluded.

Immune Approaches to Amyloid Disruption

Antibodies binding the A β N-terminal region had been shown to disaggregate fibrillar amyloid *in vitro* [5]. The unequivocal demonstration by Schenk *et al.* [6] that immunizing a transgenic (Tg) mouse overexpressing a mutant amyloid- β precursor protein (A β PP) with A β 42 could abolish or reduce amyloid pathology, initiated an extraordinary sequence of events that culminated in the vaccination of humans with early clinical signs of AD. This simple and counterintuitive therapy prevented or disrupted A β deposits and the generation of dystrophic neurites as well as the reactive astrogliosis typically distributed around senile plaques. Experiments with PDAPP Tg mice, aged to allow substantial amyloid deposits to form prior to vaccination, revealed that plaque burdens and pathology were significantly reduced, suggesting the treatment both disrupted and actually cleared the extant amyloid pathology from the brain.

Extending the vaccination strategy of Schenk *et al.* [6] to a variety of Tg mouse models resulted in the rapid and repeated demonstration of successful remediation of amyloid deposit pathology and reversal of cognitive deficits [7-10]. This response was also attainable by the simple passive infusion of antibodies in the periphery, suggesting that a fraction of the provided immunoglobulins crossed the blood-brain barrier to act in the brain tissue directly [11].

Human Ad and Animal Model Differences

The spectacular successes following Tg mouse A β vaccinations led to an extraordinarily rapid extrapolation to the human condition and clinical trials. But substantial differences exist between mice and humans in terms of lifespan, basic physiology and neuronal network complexity. Rodent neurons may exhibit greater resistance to stresses such as hypoxia and ischemia and assuming functional equivalence in responses between Tg mice and AD patients carries some risk. For example, mice have more than 75 known protease genes than humans even though the mouse genome is 14% smaller than the human genome [12,13]. In light of the fact that few comparable primate vaccination studies have been undertaken [14,15] and the second generation of clinical trials is already underway, we hope this well intended leap is successful.

Stained amyloid deposits created in many Tg mice bear a remarkable morphological resemblance to the senile plaques found in AD patients and recreate some of the neuropathology observed in AD. However, the systematic analysis and comparison of several mice strains engineered to overexpress human A β PP genes has revealed considerable heterogeneity between the various models in the expression of amyloid pathology [16-22]. Transgenic animals produce amyloid peptides that generally lack extensive post-translational modifications and are substantially more susceptible to disruption than A β species characteristic of human senile plaques. Whether these differences reflect the vastly different pace of amyloid evolution forced in Tg animals or more subtle underlying differences in biochemistry between human and rodent is unclear [18].

The Tg PDAPP mice over-expressing the mutant familial AD (FAD) A β PP 717 Val $\rightarrow$ Phe gene [23] have been widely employed as a model for amyloid deposition pathology. This rare

mutation results in an early onset and aggressive dementia characterized, besides the generation of A β 40 and A β 42, by the production of longer A β peptides and unique flocculent plaques with a near total absence of classical senile plaques found in sporadic AD [24]. Detailed biochemical examination revealed PDAPP mice produce a unique, blended amyloid deposit pathology that is neither duplicated by other A β PP transgenic mice nor precisely characteristic of either FAD or sporadic AD [20]. Amyloid- β -like molecules with C-termini extending beyond the typical 40-42 amino acid end points as well as peptides with N-terminal truncations that are more soluble than sporadic AD species are produced in PDAPP mice [20]. In addition, A β PP proteolytic processing activities are altered resulting in the lack of normal fragments C-terminal (CT)83 and CT99 [20]. Despite these unique features, the rough equivalence in response of senile plaque structures to vaccination observed between PDAPP mice and AN-1792 vaccinated patients suggests these mice are a useful and practical model for evaluating treatments to reduce or prevent amyloid deposits in humans at the earlier stages of Alzheimer's dementia.

Although the pace and extent of vascular amyloid deposition differs between Tg mouse models [16-22], the amyloid present in this compartment neatly approximates that of human AD in its resistance to disruption by immunization.

Human Vaccination Outcomes

Detailed post mortem histological and biochemical examinations following AN-1792 vaccination of humans revealed a suite of changes similar to those observed in vaccinated Tg mice [25-30]. The degree of response to vaccination varied both according to the specific pathologic lesion and physical location.

a. Senile Plaques

Senile plaques were the most sensitive to immunologic disruption, with diffuse deposits apparently less susceptible and cerebrovascular amyloid refractory to this treatment [25-28]. These differential responses undoubtedly reflect the underlying amyloid species composition characteristic of each deposit type. Senile plaques were disrupted but not completely eradicated, with Ferrer *et al.* [26] observing the presence of abnormal “collapsed” plaques surrounded by microglia and multinucleated giant cells containing A β 40/42 deposits. Nicoll *et al.* [29] observed “moth-eaten” plaque remnants and sparse residual dense plaque cores in three vaccinated patients while Patton *et al.* [28] described regions harboring possible plaque debris characterized by a small, central punctiform core surrounded by a halo of amyloid. The clear zone between core and halo exhibited no binding of anti-A β antibodies. Although the total amount of recoverable SDS-insoluble amyloid material was reduced substantially as a consequence of vaccination, MALDI-TOF mass spectrometry (MS) analysis of this material, that may correspond to the fibrillar remnants of collapsed plaques, revealed it contained a complex array of A β -derived peptides [28]. Senile plaque structure is complex [31] and it is possible that the A β remnants still microscopically observable in collapsed plaques simply represent a fraction of deposited amyloid that is associated with heterologous ancillary molecules and inaccessible to antibodies.

Interestingly, the vaccine-mediated disruption of senile plaque deposits was accompanied by a sharp increase in the amounts of water soluble A β 40/42 present in the gray matter compared to the levels found in a group of sporadic AD patients [28]. This pool represents plaque-associated A β monomers and oligomers as well as molecules free in the interstitial fluid or cytosol. MALDI-TOF MS (Matrix-assisted laser desorption/ionization mass spectrometry) characterization revealed the presence of A β -derived peptides in this extract [28]. Even though remarkable senile plaque regression was observed in vaccinated patients, ELISA analyses revealed that the total A β levels in the brain gray matter did not exhibit a correlated and

proportionate decrease. Instead, A β levels of vaccinated patients were increased in comparison to the ranges previously observed in AD patients [28]. This failure of disrupted A β to exit the brain was also observed in Tg mice [7,32]. MALDI-TOF MS analyses revealed that all the various amyloid-containing compartments, soluble, SDS-insoluble, vascular and WM, harbored a complex array of probable A β peptide-derived species exhibiting a variety of truncations at both the amino- and carboxyl-termini. Despite hydrolytic processing, these degraded remnants linger within the brain tissue and total net amyloid egress in these patients was apparently negligible.

b. Diffuse Amyloid

Diffuse amyloid deposits also exhibited a general, although not universal, pattern of focal depletion in response to AN-1792 vaccination [26-28]. In contrast to the reaction of senile plaques common in immunized patients [27,28], there was an apparent variable persistence of diffuse plaque structures. Patton *et al.*, [28] were able to isolate substantial amounts of the most insoluble P3 component composed of A β 17-42 from both of two examined vaccinated cases. Studies of an AN-1792-immunized patient cohort by Hock *et al.* [33], revealed recognition of diffuse A β and vascular deposits, but the most avid binding activity was directed against senile plaques. An epitope mapping study of sera obtained from AN-1792-immunized patients, producing significant total titers, revealed that the induced antibodies primarily recognized the first ten N-terminal residues of the A β peptide [34]. This antibody binding profile in which the bulk of the antibody binding sites were located at the amino terminus of A β 42 may partially explain the greater recalcitrance of diffuse amyloid deposits to disruption. N-terminal truncation of amyloid peptides deposited in human AD patients is substantial, suggesting the therapeutic benefits of antibodies targeting the amino terminus of A β would be limited [16, 19]. Moreover, studies of antibodies produced in A β 42 vaccinated mice directed against residues 4-10 primarily recognized amyloid plaques extant in human AD brain samples [35].

c. Vascular Amyloid

Substantial amounts of vascular amyloid persisted in senile plaque-depleted regions in every vaccinated patient examined [25-29]. Even though cortical and leptomeningeal vascular amyloid was resistant to disruption, vaccination produced substantial changes in these deposits which exhibited an apparent increase in severity and molecular composition complexity [25, 28]. The elevated cortical and leptomeningeal vascular A β 42 levels [26,28], combined with the presence of an uncharacteristically rich spectrum of A β degradation products revealed by MALDI-TOF MS suggests the amyloid molecules mobilized out of senile plaques were subsequently retained in the vasculature. Given its intrinsically greater toxicity, the long-term effects of shifting the balance of vascular amyloid pools toward A β 42 and/or degraded or post-translationally modified species may be deleterious.

d. White Matter

The WM does not exhibit the spectacular pathologic lesions such as senile plaques and neurofibrillary tangles (NFT) found in the gray matter, but this crucial compartment is affected in AD and was drastically altered after AN-1792 vaccination. Nicoll *et al.*, [25] noted substantial WM atrophy and pathologic inflammatory alterations and Ferrer *et al.*, [26] found extensive demyelination in the frontal lobe WM in a vaccinated patient. A broad range of structural changes in WM is a common feature of AD [36,37]. Although no pathological alteration in the WM is directly attributable to A β , elevated levels of soluble oligomers or other species are present in AD patients, suggesting that this compartment is a sink for A β synthesized in the gray matter or elsewhere [36]. The total A β 40 and A β 42 increased dramatically in vaccinated patients to amounts far beyond the elevated levels typical of sporadic AD cases. The fact that MALDI-TOF MS experiments revealed an extraordinary range of probable

amyloid-derived species, many of which exhibited post-translational modifications like those of cortical A β , suggests that the WM was an ultimate sink for mobilized senile plaque molecules. Given that the majority of sporadic AD patients exhibit a broad range of profound pathological changes in WM structure [25,26,36,37], this outcome is worrisome.

e. Tau and NFT Pathology

The AN-1792 vaccination induced marked changes in amyloid deposition patterns, but exerted a comparatively minor impact on *tau*-mediated pathologic lesions [25-29]. Ferrer *et al.* [26] and Nicoll *et al.* [29] noted that while areas with intact neuritic plaques also harbored phosphorylated *tau* similar to that found in AD patients, amyloid plaque-depleted regions contained collapsed plaques with almost no *tau*-reactive neurites, but NFT and neuritic threads were unaffected and still present. Additional postmortem examinations conducted by other groups noted a similar pattern of regions in which amyloid plaques and diffuse deposits were apparently depleted while NFT remained in place, largely untouched in the face of such drastic amyloid pathological lesion alterations [27,28]. Triple Tg mice exhibiting both plaques and NFT subjected to anti-A β immunotherapy or a γ -secretase inhibitor revealed marked *tau* and amyloid deposit reductions. However, remediation of *tau* pathology by these methods was only partially effective, with hyperphosphorylated *tau* aggregates appearing impervious to disruption [38]. Immunotherapy of the same Tg mice with an anti-*tau* antibody failed to exert any detectable effects on *tau* or A β pathology [38].

Individuals immunized with AN-1792 exhibited mild to moderate dementia, meaning that this intervention occurred relatively early in the clinical progression of AD. In spite of early application of immunotherapy and the dependence of *tau*-associated pathology on A β , this intervention was not quick enough to prevent an effectively irreversible NFT evolution. Neurofibrillary tangles and neuropil threads are correlated with the cognitive impairment of AD and the persistence of these lesions in the face of anti-A β vaccination has led to suggestions that earlier interventions may be more efficacious [39]. Certainly the tenacity of these dementia-linked lesions complicates clinical assessments of cognitive improvements that follow anti-amyloid immunotherapy. Perhaps unless vaccination is offered before NFT become numerous, the best outcome associated with this therapy could be a marked slowing or halting of dementia progression.

Adverse Events and New Animal Model Experiments

Although vaccinating PDAPP and other Tg mice against A β initially appeared successful and without overt toxicity, the rapid development of aseptic meningoencephalitis in some of the AN-1792-vaccinated patients led to an early suspension of that trial [1]. Later studies examining both active and passive immunization revealed the potential for the development of reactive autoimmune encephalomyelitis in mice [40,41]. Given this lack of equivalence between humans and mice, examinations of other model systems have been undertaken. Non-human primates may provide more faithful models of the type of immune responses elicited in aging AD patients [14]. Studies of Caribbean Vervets revealed an age-dependent deposition of amyloid and associated neurodegeneration which was subjected to modulation by vaccination [15]. Canines accumulate diffuse amyloid plaques with age and exhibit correlated cognitive declines and a recent long-term study of A β 42-immunized dogs revealed deposits were reversed, but found no improvement in cognition [42]. The authors suggest that earlier intervention before amyloid pathology is developed may result in an improved cognitive status. This outcome parallels the results with AN-1792-vaccinated humans in which evidence for decisive cognitive improvements is equivocal.

Vaccination and Cognition

A follow-up study of AN-1792-immunized patients confirmed to generate anti-A β antibodies showed these patients exhibited significantly slower decline in cognition and functional decline rates [43] while a 12 month follow-up study conducted by Gilman *et al.* [44] was not as clear. A recent long-term study of eight select AN-1792-immunized patients who died with AD suggests that although vaccination disrupted amyloid plaques, this therapy did not enhance cognitive function or necessarily halt neurodegeneration [30]. Volumetric MRI studies of AN-1792 vaccination responders revealed that this group had significantly greater volume decreases and ventricular enlargement than control patients, although the losses were not correlated with more severe cognitive declines [45]. A later study specifically investigating the relationship between A β burden and brain atrophy found that total A β levels were not linked to rates of brain loss or ventricular expansion [46]. The fact that AD dementia is a complex melange of underlying toxic effects ascribable to both amyloid and NFT pathology, as well as other idiosyncratic factors related to aging, confounds any simple interpretation of an experimental outcome.

Assessing the Case for Early Intervention

The intricate involvement of vascular pathology in AD evolution and the firm recalcitrance of vessel-associated amyloid to antibody-mediated disruption suggest that immunization therapies would be most effective if administered on a prophylactic basis before vascular impairment and well ahead of any clinically evident cognitive decline. The results of experiments with Tg mice and the first clinical trial clearly support this general idea [6,7,11, 25-29,47]. Deploying anti-A β vaccines prior to the onset of any evidence of dementia is not feasible at present. However, Klunk *et al.* [48] have pointed out that FAD gene carriers represent an extraordinary valuable resource for the clinical evaluation of amyloid disrupting therapies. If voluntary and preemptive vaccination of known FAD gene carriers either staves off the onset of dementia or prevents it in such patients, the case for similar approach against sporadic AD will be virtually irresistible. Alzheimer's disease pathology is presumed to require extended periods to develop, but recent studies in Tg mice suggest that under the proper conditions senile plaques seed quickly [49]. Pathological signs of this emergence may be too subtle to be easily detected, increasing the need for preventative therapies. Preliminary results from phase II clinical trials with the humanized monoclonal antibody bapineuzumab suggest that *apolipoprotein E (ApoE)* genotype influences safety and efficacy (Alzheimer Research Forum <http://www.alzforum.org/new/detail.asp?id=1850>). Carriers of *ApoE* $\epsilon 4$ allele had more serious adverse events and did not exhibit clear cut improvements. These findings underscore the complex nature of AD pathology and the risks inherent in preventative therapies ultimately applied to a diverse population.

Amyloid- β is viewed as pathological based on the postmortem correlation of senile plaques with an AD diagnosis. However, the recent follow-up study of eight AN-1792-immunized patients of Holmes *et al.* [30] suggests that senile plaque disruption may not halt disease progression. It remains uncertain which of the various forms of this peptide is the most toxic and whether A β performs any desirable or protective functions. Perhaps A β molecules maintain the vascular integrity with or without recourse to blood coagulation by functioning as rapid sealants and thereby prevent conditions that could lead to neuronal injury [50]. This suggests that immunotherapy that is too effective at removing and/or preventing A β deposits could pose risks to the vasculature, an outcome that has been observed in immunized Tg mice [51,52]. In APP23 and the double transgenic APP/PS1 mice which produce a substantial vascular amyloid pathology, anti-A β immunotherapy resulted in increased rates of micro-hemorrhages [51,52]. The long-term cognitive effects of chronic immunotherapy producing a steadily accumulating and effectively permanent pool of disrupted A β peptides within the human brain are unknown.

The fact that vaccination of Tg animals produces measurable cognitive improvements [7,32, 53], despite the persistence of A β , is of relevance to the human condition only if rodent and human neurons react to amyloid and vascular pathology in an equivalent manner. It must be remembered that the Tg mice amyloid deposition models lack the full spectrum of biochemical and pathological conditions that will co-exist in the typical human AD patient. This complicates all attempts to extrapolate experimental results in animals to the human condition directly.

Amyloid- β released from plaques apparently did not exit the brain of immunized patients [28], paralleling results obtained with some Tg mice models [7,47]. If senile plaques are actually a mechanism to sequester potentially toxic A β molecules [3,4] disturbing these structures may pose unanticipated risks over the long term. Despite its strong conservation over great phylogenetic distances [18], the physiological functions of A β are still unclear. A largely neglected corpus of evidence compiled and reviewed by Heininger [4,54] calls attention to the potent physiological and metabolic effects exerted by A β and their potential adaptive significance to a brain confronting age-related circulatory insufficiency, energy deprivation and consequent metabolic stress. Active A β immunotherapy perturbed fundamental tissue fluid dynamics to such an extent that unprecedentedly high levels of A β were detected in the white matter [28]. As it stands, immunotherapy may not represent an AD cure; it simply changed the physical state of A β and transferred a considerable fraction of the disrupted amyloid deposit molecules to different brain compartments [28].

Approaches utilizing passive immunization have been examined in clinical trials. Intravenous immunoglobulin (IVIg) contains anti-A β antibodies and a short term study in a small number of early stage patients revealed proportionately increased levels of both specific antibodies and A β in plasma [55]. In addition, cerebrospinal fluid A β levels decreased with administration of IVIg and fluctuated in step with the presence of exogenous anti-A β antibodies. Most important, Mini-Mental State Examination scores increased with initial IVIg administration, returned to original levels as A β antibodies declined and stabilized with additional IVIg infusion. This was a short term and small scale study that will require further investigation.

Although antibodies are therapeutic tools of extraordinary precision, inducing or administering immunoglobulins in the periphery to effect a change within the CNS is considerably less focused. The side effects of such therapy, which could extend far beyond the brain, are uncertain. A concerted effort to detect and evaluate long-term adverse reactions in the periphery must be undertaken in Tg mice and other models. Given the potential of A β to impact a substantial range of processes, it seems wise to scrutinize all study participants for their state of health across many parameters beyond simply seeking to assess cognitive status. One issue that must be clarified is the overall level of amyloid in the periphery to act as a sink for antibody. Since only a small fraction of the immunoglobulin present in the circulation will ever enter the brain, maintaining high total antibody titers for the longest possible intervals is essential and may be confounded by the presence of sizeable antibody sinks at other locations.

Dementia is a relentlessly progressive process of multiple years duration that is only partly correlated with senile plaques. The fact that certain FAD patients exhibit aggressive and early-onset dementias with amyloid deposition patterns that differ from sporadic AD [56] further emphasizes the comparatively weak link between senile plaques and cognitive impairment. In addition, postmortem examinations revealed consistently that NFT pathologic alterations were unaffected by A β vaccination [25-29]. Taken together, AN-1792 follow-up studies of immunized patients have suggested that even extensive senile plaque disruption might yield at best only modest and temporary clinically quantifiable cognitive improvement [30,43,44]. These findings remind us to take a more synthetic view of A β and the amyloid cascade as one aspect of a complex and many-faceted process that ends in AD dementia. Assessment of the outcome of vaccination will help clarify the importance of the amyloid cascade to AD dementia.

Although many recipients of the original vaccine have now survived for years, passive immunotherapy performed on a chronic basis promises even more effective amyloid disruption across a greater range of patients. This approach to disrupting amyloid deposits may well be efficient, but only time will reveal if it is wise.

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FAD	Familial Alzheimer's disease
IVIg	Intravenous immunoglobulin
MALDI-TOF MS	Matrix-assisted laser desorption/ionization mass spectrometry
NFT	Neurofibrillary tangles
Tg	Transgenic
WM	White matter