

Alzheimer's disease: Toward the rational design of an effective vaccine.

Enfermedad de Alzheimer: Hacia el diseño racional de una vacuna eficaz.

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SUMMARY

The promising clinical results with the human monoclonal antibodies aducanumab and solanezumab targeting β -amyloid in Alzheimer's disease treatment, confirm both the amyloid cascade hypothesis and protective natural immunity, while strengthening the immunotherapeutic approach. That aducanumab recognizes a conformational epitope formed by oligomers emphasizes the need for whole β -amyloid, not just its B-cell epitopes as have been the norm to avoid pro-inflammatory Th1-reactions. That truncated β -amyloid having N-terminal pyroglutamate is present only in diseased brain implies a new useful vaccine antigen. Another relevant antigen is the tau protein, which shows a close association and cooperativity with β -amyloid in exacerbating this disease. Hence, effective vaccines may be polyvalent, presenting to the immune system a number of antigens relevant to induce an immune response to prevent or slowdown the onset of this disease. The presence of both B and T cell epitopes in the antigens, require a sole Th2 immunity to avert brain inflammation; a task that cannot be attained with adjuvants that under any conditions induce Th1 and/or Th17 immunities. Hence, new vaccine adjuvants are needed to safely induce Th2 while inhibiting Th1 immunity, an objective that can be achieved with certain fucosylated glycans or triterpene glycosides, which apparently bind to the DC-SIGN lectin on dendritic cells polarizing the immune response toward Th2 immunity. Because the triterpene glycosides have the pharmacophore needed to co-stimulate T cells, they may ameliorate the T-cell anergy associated with immunosenescence and responsible for poor vaccine efficacy in the elderly population, a critical issue for an Alzheimer's vaccine.

KEYWORDS: Alzheimer's, immunotherapy, vaccines, adjuvants, immunomodulators

RESUMEN

Los resultados prometedores en el tratamiento de la enfermedad de Alzheimer con Aducanumab y Solanezumab, anticuerpos monoclonales humanos contra β -amiloide, ratifican la hipótesis de la cascada del amiloide y la existencia de inmunidad natural contra Alzheimer, mientras refuerza el método inmunoterapéutico. Que Aducanumab reconoce un epítipo conformacional formado por oligómeros, acentúa la necesidad del β -amiloide completo y no solo sus epítipos de células B, como ha sido la norma para evitar reacciones pro-inflamatorias Th1. De que el β -amiloide truncado con piroglutamato en su extremo N-terminal se encuentra solo en cerebros enfermos, es un antígeno útil; otro antígeno importante es la proteína tau, que tiene una estrecha asociación y cooperatividad con β -amiloide en exacerbar esta enfermedad. Una vacuna eficaz puede ser polivalente para presentar al sistema inmunológico una variedad de antígenos importantes e inducir una respuesta para prevenir o retardar el comienzo de esta enfermedad. La necesidad de epítipos de células B y T en los antígenos, implica una inmunidad tipo Th2 para evitar inflamación del cerebro; objetivo que no se puede alcanzar con adyuvantes que inducen inmunidades Th1 y/o

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Th17. Consecuentemente, se necesitan nuevos adyuvantes de vacunas para inducir sin riesgos la inmunidad Th2 mientras se inhibe la Th1, objetivo que se puede lograr con ciertos glicanos fucosilados o glucósidos triterpénicos que se unen a la lectina DC-SIGN en células dendríticas, polarizando la respuesta hacia la inmunidad Th2. Como los glucósidos triterpénicos tienen el farmacóforo necesario para co-estimular las células T, podrán moderar la anergia de las células T asociada con inmunosenescencia, responsable por la baja eficacia de las vacunas en la población anciana, materia crítica para vacunas contra la enfermedad de Alzheimer.

PALABRAS CLAVE: Alzheimer, inmunoterapia, vacunas, adyuvantes, inmunomoduladores.

INTRODUCTION

After decades of failures in the treatment of Alzheimer's disease (AD), the promising clinical results obtained during a phase Ib trial with the human monoclonal antibody (mAb) aducanumab, from Biogen (1), have brought optimism while showing that the immunotherapeutic approach is apparently the most promising one to prevent/treat this disease. Indeed, those initial results were supported by the subsequent clinical studies with solanezumab (Eli Lilly), a humanized mAb that binds the central epitope of monomeric A β , preventing its aggregation and slowing the disease progress (2). Those results besides validating the immunotherapy's method, also confirm the "beta-amyloid cascade hypothesis", which for years has provided the scientific bases to explain this disease and develop drugs to treat it (3); a notion that due to the numerous clinical failures, has been questioned. These results' relevance is emphasized by the current number of worldwide AD cases, about 40 million, which is expected to be over 80 million by year 2040, and after double every 20 years (4); obviously an outcome of the increased longevity as a result of modern medicine. Hence, this alarming raise of this worldwide epidemic demands near term solutions, to prevent and/or delay the onset of AD. While various approaches to develop drugs for AD are being pursued, those based on a protective immunological response are the most sound, i.e. studies have shown that humans at an early age start to produce antibodies against the protein beta-amyloid (A β), a response that decreases with age as the incidence of AD starts to increase (5). The presence of these protective anti-A β antibodies has been confirmed by the studies with aducanumab, which is a replica of antibodies present in older by mentally sound human beings (1). Yet, this immune response also occurs in non-humane primates and other animals like dogs and cats, which show a neurodegenerative process similar to AD (6,7). While there are rare cases where AD is due to mutations of

the A β gene, this disease or sporadic AD is largely a result of the aging process.

The A β cascade hypothesis

Germane to AD is the A β cascade hypothesis (ACH), which over twenty years ago proposed that the deposition of aberrant A β aggregates as plaques on neuronal cells was the primary cause of their death. Since then and because brain damage was observed before plaque formation and other findings, the hypothesis has gone through several iterations that include A β soluble forms, which are also toxic to brain cells (3,8). In fact, A β shows a complex cascade of conformational and oligomerization stages that lead to the formation of neurotoxic forms, which may involve other proteins (Figure 1). A β consists largely of two isoforms, a main peptide called A β 40 that has amino acid residues from 1 to 40 and a minor one that is less than 5% of the A β , which has 2 extra amino acids and is named A β 42. While both isoforms have a tendency to form β -sheets, because A β 42 has more aggregability than A β 40, it seems that it starts the process leading to the formation of oligomers, fibrils and plaques (9). A feature of A β is the unique spatial structures formed by its aberrant aggregates, i.e. conformational epitopes that are independent from its amino acid sequence. Indeed that proteins classified as amyloids, A β being one of them, while they do not share amino acid sequence homologies have a similar generic conformational epitope, has been shown using mAbs (10). Of interest is that these proteins are frequently associated with amyloidosis, a group of diseases characterized by protein misfolding or proteinopathies (11,12).

The products resulting from the changes taking place at the A β cascade are this protein's aberrant conformations and oligomers, showing different degrees of stability and cytotoxicity. Since A β belongs to the amyloid proteins, i.e. small proteins lacking

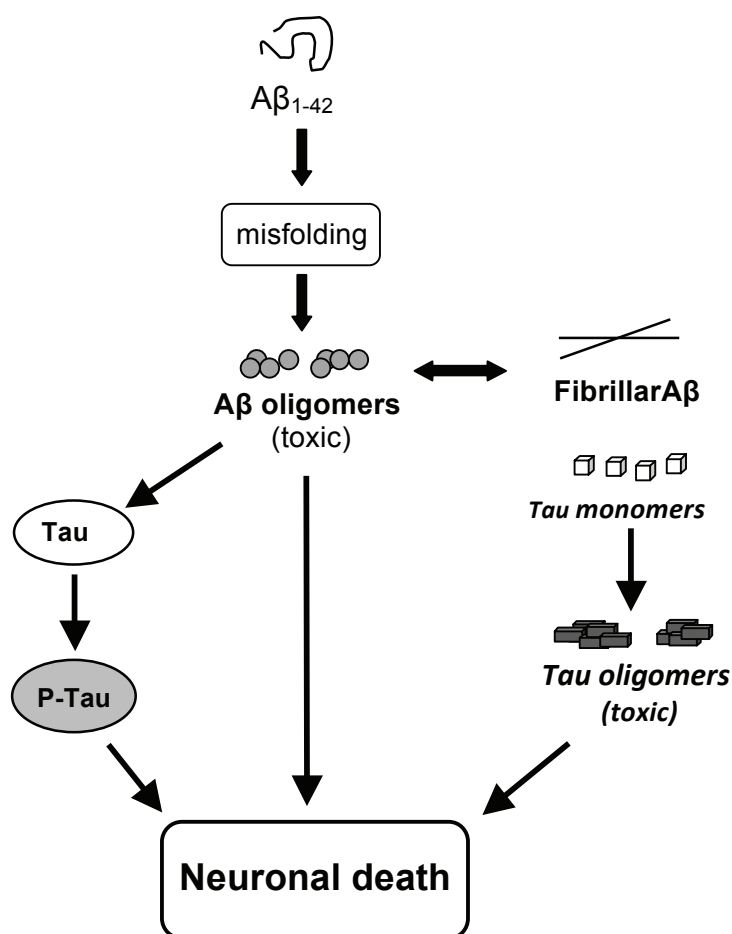


Figure 1. Description of some events associated with the ACH. The misfolding of monomeric Aβ₁₋₄₂ leads to the formation of toxic Aβ soluble oligomers and insoluble fibrils. Toxic Aβ oligomers cause synaptic damage, killing the neurons by different mechanisms, and inducing hyperphosphorylation of tau (P-Tau), which leads to neuritic dystrophy. Tau alone can also form toxic oligomers that kill the neurons without being hyperphosphorylated. A result of the injuries caused by these two proteins is neuronal death, which causes dementia or AD. Protective mAbs and Nabs recognize conformational epitopes present in toxic Aβ and tau oligomers, removing them.

ordered structures and with tendency to form amyloid fibrils rather than crystals; its assembly is complex and diverse, which can be affected by the presence of other proteins and lipids, and is characterized by extensive polymorphism (12). Following extracellular release of the Aβ monomer and its interactions with GM1 ganglioside or either ApoJ or αB-crystallin proteins, it forms a variety of pre-fibrillar soluble Aβ oligomers that differ in size and are more cytotoxic than fibrillar Aβ (13). Hence, it's now well established that soluble Aβ oligomers are more relevant for AD than fibrillar Aβ; indeed cytotoxic Aβ oligomers can kill the cells by a variety of mechanisms, finding that pinpoints to

these forms as the therapeutic target rather than the fibrillar forms. This proposal is supported by the prion diseases, where misfolding of amyloidogenic peptides lead to neurotoxicity without plaque formation (14).

Though the aducanumab and solanezumab results strongly support the crucial role of Aβ in AD pathology, proteins like tau may have also a role in this disease. In effect, tau is affected *in vitro* by subnanomolar concentrations of soluble Aβ dimers isolated from the brains of AD patients, which induce its hyperphosphorylation that disrupts the microtubule cytoskeleton, causing neuritic dystrophy; an effect that

Table 1. Alzheimer's disease vaccines that have completed or are currently undergoing clinical studies.

VACCINE	ADJUVANT	ANTIGEN	SPONSOR	STATUS
AN1792	QS-21 (Th1)	A β ₁₋₄₂	Elan/Wyett	ended
Vanutide cridifar	QS-21 (Th1)	A β ₁₋₇ /CRM197	Pfizer/J&J	ended
V950	Iscomatrix (Th1)	A β peptides	Merck & Co	ended
CAD106	None	A β ₁₋₇ /VLP bacteriophage Q β	Novartis	Phase 2
UB311	CpG ON (Th1)	A β ₁₋₁₄ /UBI Th	UBI	Phase 1
Affitope AD02	Alum (Th2)	A β ₁₋₆ /KLH	AFFiRiS/GSK	ended
ABvac40	Alum (Th2)	Short C-terminal peptide/KLH	Grifols SA	Phase 1
AADVAC1	Alum (Th2)	Tau peptide/KLH	AXON Neuroscience	Phase 1

can be blocked by anti-A β antibodies (8). In fact, earlier *in vivo* studies showed that intra-cerebral infusion of young mice with A β containing brain-extracts from aged mice induced tau pathology in APP x Tau transgenic mice (15). As both proteins are physically in close contact and have significant interactions; it is very possible that they may act in a concerted manner, magnifying their pathological effects (Figure 1). Proof for the interactions between A β and tau is provided by studies using a mouse model with a genetic knock-out for tau protein, which showed that absence of tau in knock-out mice prevents the synaptic dysfunction caused by acute exposure to A β 42 (16) and that tau acts downstream of A β , as proposed by Hardy and Selkoe. Indeed, tau can exert its neurotoxicity alone or in a cooperative manner with A β . While it was assumed that only hyperphosphorylated tau was neurotoxic, new evidence shows that like A β , tau oligomers rather than the fibrils are the toxic forms regardless of their phosphorylation (17). Hence, due to the close interactions among A β and tau in AD pathology, an option would be to have both proteins as therapeutic targets, as have been suggested (18). While the functions of A β and its precursor protein, APP, are being elucidated, apparently their normal forms play a role in neural cell development and survival, plus enhancing memory; functions apparently dependent on the protein folding and concentrations. Thus, therapeutic strategies should distinguish the good from the bad protein species, to prevent complications due to therapy.

Immunity and Alzheimer's disease

Several studies have shown the production by healthy people of autoantibodies (Nabs) against A β , production that starts at a very early age and that after being maintained through most of the life, begins to decrease with aging and is quite lower in AD. That the NAbs' decrease occurs when AD starts to develop implies a neuroprotective role of immunity against AD. In effect, a retrospective study had shown that previous treatment with intravenous immunoglobulin (IVIg) reduces the risk of AD by 42% (reviewed in 19), significant when it is considered that the donor population is made of young, healthy adults that supposedly have higher levels of A β antibodies. Later studies showing that NAbs react with oligomeric but not monomeric A β , imply that these antibodies by targeting toxic oligomers rather than the non-toxic monomers protect against AD. One study also showed that delivery of NAbs to an AD mouse model reduced plaque formation and led to improvement of the mice behavior, confirming these antibodies' protective role against AD (20). As NAbs recognize oligomeric but not monomeric A β , it is evident that they recognize a conformational epitope that must be common to the different oligomers. Because this epitope is independent of A β 's amino acid sequence, but dependent on the new spatial structure shaped by the assembly of several monomers into oligomers, its presence would require the whole protein molecule and not just some partial peptides.

Convincing support for the protective anti-A β Nabs is provided by the results of the passive immunotherapy study with aducanumab, a human recombinant IgG1 mAb cloned from aged donors that were cognitively normal and thus have effectively resisted AD. Like protective Nabs, this mAb binds selectively and with high affinity to A β soluble oligomers and insoluble fibrils, but not to monomers (1). Although there is no information about the nature of the epitope recognized by aducanumab, as it only recognizes aggregated forms of A β , it is most likely a conformational epitope like that described for Nabs. From this clinical study and previous animal studies, though not well understood it is clear that the natural autoimmune response differentiates between “good monomers” and “bad oligomers” of A β ; which suggests a biological role for monomeric A β . While several mechanisms have been proposed to explain the antibody-mediated removal of A β , the one for aducanumab seems to involve microglial-mediated phagocytosis and clearance of A β through the IgG1, which largely depends on the effect or function of the mAb (1). This mechanism requires passage of the antibodies across the blood-brain barrier (BBB) and into the CNS, an immune privileged site; passage that due to the BBB would be limited, which would explain the high doses of this mAb needed to achieve a therapeutic effect, as compared to the doses needed for mAbs acting outside the CNS.

Although the focus of passive immunotherapy has been the IgG immunoglobulins, IgMs also play a role in the removal of A β , but by a different mechanism that does not involve either microglia or macrophages. The IgMs exert their anti-A β activity by catalytically degrading this protein, i.e. these antibodies are highly specific proteases that recognize A β at concentrations lower than those required for IgG binding (21). As due to their size, ~ 900 KDa, it is unlikely that IgMs would pass across the BBB, they act by degrading the peripheral A β that has been transported across the barrier as a complex with anti-A β IgGs. That the content of catalytic IgMs, relative to the other antibodies, increases with age points to their aging-induced synthesis that is present even in those affected by AD; probably to maintain the capacity to process the increasing amounts of toxic A β forms associated with aging. Due to their relatively long life in circulation (half-life approximately 5 days), coupled to the fact that one IgM can process several A β molecules, catalytic IgMs can be effective therapeutic agents for AD (22). Yet, catalytic IgMs are not limited to AD, but they are also present in other

autoimmune conditions, apparently a quick protective response against aberrant autoantigens. Since the IgM response is T-cell independent and devoid of memory T-cells, it is unlikely that these catalytic properties would be passed to IgG antibodies after the IgM \rightarrow IgG class switching; indeed, natural anti-A β IgG preparations and IgG mAbs have negligible proteolytic activity. Hence, catalytic IgMs can be considered an innate immunity rather than an adaptive immunity mechanism.

While an anti-A β antibody response or humoral immunity is usually associated with positive effects on the prevention or slowing down of AD, a pro-inflammatory response or T-cell immunity is usually linked to damaging effects on the CNS. Although humoral immunity is linked to one type of T helper cells, i.e. Th2, the pro-inflammatory immunity is linked to two different types of T helper cells, i.e. Th1 and Th17. In Th2-biased immunity, besides stimulation of antibody production, there is production of anti-inflammatory cytokines, e.g. IL-4 and IL-10; while Th1-biased immunity is characterized by the pro-inflammatory cytokines, e.g. IL-2 and IFN- γ , and the production of several effector cells, like CD8+ T cells or cytotoxic T lymphocytes (CTL), activated macrophages and others. A new type of T helper cell is Th17, which induces an inflammatory immunity mediated by pro-inflammatory cytokines IL-17 and IL-22; while Th17 immunity provides a strong anti-microbial response at the mucosal barrier, it is often associated with damaging inflammatory responses and tissue injury in several autoimmune conditions, e.g. multiple sclerosis and rheumatoid arthritis (23). That some agents that elicit Th2 immunity can induce Th17 in the absence of Th1 immunity, is an important issue in the design of AD vaccines.

While there is consensus that in AD an inflammatory immunity is mostly damaging, animal studies have delivered different mechanisms to explain such immune response; apparently the result of factors like the animal species and/or their different strains used in the studies, as previously reported. Still, a variable that can significantly impact those results is the adjuvant or immune modulator used to stimulate an immune response; i.e. most studies assume that all Th1 adjuvants eliciting an inflammatory response have similar mechanisms of action. But, the evidence from infectious diseases and cancer vaccines shows that such is not the case. Actually, although almost all of the known adjuvants induce Th1 immunity, a result of the induction of adaptive immunity following the

stimulation of innate immunity, their mechanism of action is dependent on the innate immunity receptor involved, like the toll like receptors (TLRs) (24). Proof of these different mechanisms is provided by the fact that concurrent stimulation of different TLRs, results in a synergistic effect on the immune response due to interactions between different and independent immune modulatory pathways. This situation may explain the different results from animal studies, and the failures in developing an effective AD vaccine for humans.

Though a discussion of inflammation and AD is beyond this review, some issues relevant to active immunotherapy will be briefly addressed here. While accepted that inflammation has a damaging role in AD, as it involves highly interacting molecular mediators and mechanisms, some of them beneficial, plus the lack of a clear insight about this response, it may be difficult and even risky to broadly interfere with it. Still, findings like A β 's intrinsic capacity to induce astrocytes and microglia to secrete *in vitro* and *in vivo* pro-inflammatory cytokines, like IL-1 α , IL-1 β , IL-6 and TNF- α , are important to develop immunotherapeutic methods (25). This pro-inflammatory activity may be explained by A β being an endogenous "danger-associated molecular pattern", which binds to innate immunity receptor(s) on astrocytes and microglia, to initiate an innate immune response that triggers an adaptive inflammatory response. Indeed, A β induces *in vitro* the expression by microglia of TLR-2 and TLR-4, TLR-2 being the microglia's primary receptor for A β ; binding of A β to TLR-2, would initiate a neuroinflammatory activation, which could explain some of A β damaging effects(26). Another inflammatory cytokine that apparently aggravates AD is IFN- γ , i.e. A β -specific CD4+ Th1 cells adoptively transferred to an AD mouse model increased microglia activation and A β deposition, effects that were attenuated with an IFN- γ antibody (27). Yet, this study showed that while Th2 cells had beneficial effects, the Th17 cells did not cause damage, despite being strongly inflammatory.

Evidently, different from infectious diseases and cancer, where the desired response is usually pro-inflammatory Th1, the response needed in AD is sole humoral Th2 immunity. Thus, it would be useful to induce Th2 while inhibiting, but not abrogating, the inflammatory Th1 immunoresponse; difficult as most immune modulators induce both Th1 and Th2 immunities. Thus, development of an effective and safe vaccine to prevent and/or treat AD would require

an approach closely mimicking the natural protective immunity.

Alzheimer's disease – Passive immunotherapy

The clinical results with aducanumab and solanezumab support both passive and active immunotherapeutic approaches to prevent/treat AD. In passive immunotherapy, an antibody preparation like IVIG or a mAb against an antigen, e.g. A β or tau, is administered to a patient to achieve the desired therapeutic effects. The benefit of passive AD immunotherapy is its quick effect, regardless of the recipient's immune competence; useful with the elderly that usually has an immune decline due to aging. The administration schedule and dose needed would depend on the mAb's nature; i.e. IgGs have a circulating half-life of approximately 20 days, but, due to the BBB only a small fraction enters the CNS. Yet, that ultrasound may transiently open the BBB and allow passage of antibodies from the blood into the CNS, could allow a more effective delivery of the mAbs to the brain (28). Of interest is the contrast between the clinical results from aducanumab and those from the mAbs, bapineuzumab and gantenerumab (29). Bapineuzumab is a humanized mAb that binds the N-terminal 5-amino acid residues of fibrillar and soluble A β , presumably disrupting aggregation but, it failed in clinical trials. In contrast, the human mAb gantenerumab binds to a conformational epitope made by the N-terminal and central amino acids of aggregated A β ; acting presumably by taking apart and recruiting microglia to degrade A β plaques. Yet, gantenerumab although similar in most of its properties to aducanumab, did not show clinical benefits.

It is difficult to explain the differences between the aducanumab and gantenerumab clinical results, because while there is significant information about the epitope recognized by gantenerumab (29), all that is known about aducanumab is that it is a conformational epitope found in A β soluble oligomers and insoluble fibrils, but not in monomers (1). As aducanumab was isolated directly from humans, it possibly recognizes a generic amyloid fibril epitope such as those found in other pathogenic amyloids. A characteristic of these conformational epitopes is that they are sequence independent and dependent only on the aberrant amyloids' aggregates; i.e. many misfolded amyloidogenic proteins share these conformational epitopes (30). Thus, it is possible that the body has protective antibodies against these proteins, which eliminate them before they cause damage; a protective

mechanism that decreases with age as result of immunosenescence. Indeed, amyloidosis of which AD is one of them, has been called a biological aging problem as well as a disease. Hence, the different outcomes of these studies may be due to the methods used to identify those antibodies; i.e. aducanumab was the result of an approach that took advantage of the natural selection process when they used aged, healthy and mentally competent individuals as donors (1). But, the efficacy of these mAbs may depend on various factors, i.e. solanezumab while delivered poor results in the first clinical trial (29), it gave positive results in a subsequent study (2). Yet, the presence of protective antibodies against aberrant forms of otherwise normal proteins, besides confirming the validity of passive immunotherapy, also provides strong support to vaccination as a prevention and/or treatment against AD.

Alzheimer's disease – Active immunotherapy or vaccination

Natural protective immunity indicates a possible use of passive and active immunotherapy to prevent and/or treat that disease, a notion with a long history of support from the infectious diseases and cancer areas. Hence, aducanumab and solanezumab's positive results have provided a proof that was missing in AD. While vaccination was the first immunotherapeutic approach tried in AD, a series of failures raised doubts about its feasibility as well as the science behind it. Thus, proposals to rationally develop an effective AD vaccine would need to make a retrospective analysis of past studies to identify the reasons for their failures and justify new approaches. That due to large differences between vaccines for AD and infectious agents little can be transfer to AD vaccines, can explain these disappointments, despite the presence of a natural protective immunity.

Sub-unit AD vaccines have an antigen(s) and an adjuvant or immunomodulator. While the antigen is required to induce a specific immune response, the adjuvant is the component that will stimulate and bias such a response; i.e. the adjuvant not only jumps start the immune system, but it also decides in which direction will go. As the adjuvant properties are independent from the antigen's nature, alteration of an antigen to elicit a safer immune response would not help. A concern for AD vaccines is that adjuvants generally stimulate a pro-inflammatory Th1, which is always present with a humoral Th2 immune response; i.e. adjuvants that induce a sole Th2 immunity are

rare (31). Adjuvants exert their immune modulatory activities by using different but highly specific receptors and paths to stimulate immunity; but as most immunological receptors are linked to innate immunity, e.g. TLRs and NOD-like receptors (NLRs), usually the initial innate immunity response will be followed by a pro-inflammatory adaptive immunoresponse.

The A β 42 AN1792 vaccine The first AD vaccine containing A β 42 and clinically tested, AN1792, showed in a phase 1 study a large variability in antibody response, but no side effects. While it has been reported that the phase 1 response was predominantly Th2, it is difficult to accept that conclusion since QS-21 is a Th1 adjuvant (32); hence, those results may indicate its de-acylation, which would result in Th2-biased immunoresponse. But, since during the phase 2 study, after 1 to 3 immunizations some patients developed encephalitis, the study was ended. Yet, the phase 2 antibody responders, i.e. 20%, regardless of the encephalitis, had antibodies against the linear N-terminal A β ₁₋₈ peptide; a response independent of conformation or aggregation. While the encephalitis have been rightly attributed to the adjuvant QS-21, the different outcomes of phase 1 and 2 are a result of the enhancing effects of the non-ionic detergent polysorbate 80 on QS-21's adjuvanticity (31); a difference that may have been made even more evident by a possible de-acylation of QS-21 in the phase 1 study.

Interpretation of the results from cell mediated immunity induced by the AN1792 vaccine is intricate, due to the differences in the immune response between humans and mouse models. The postmortem studies of AN1792 vaccinated patients have shown presence of CD4+T cells, CTLs and macrophage infiltration, as well as clearance of amyloid plaques, due to vaccination; yet, it is clear that A β -specific CTLs did not have a role in the encephalitis process (33). In contrast, CD4+ T cells have an important role secreting cytokines that induce inflammation; one of such cytokines with a unique position is IFN- γ , which at high levels in the brain is damaging, but at low levels helps neuronal repair. Indeed, adoptively transferred Th1-producing CD4+ T cells increase microglia activation and A β deposition that are associated with impaired cognitive function, while those producing Th2 or Th17 did not cause any changes. A question is why AD vaccines are effective in mouse models, but not in humans? A reason that has been offered is that vaccines in transgenic mice are working in a preventive mode while in humans are in a therapeutic

mode after onset of AD; i.e. it seems to be a matter of timing.

Truncated A β vaccines An outcome of the damaging results obtained with the AN1792 vaccine has been to modify the A β 42 antigen by deleting all of the T-cell epitopes, leaving only the N-terminal B-cell epitope, but mostly using Th1 adjuvants; yet, the Grifols' vaccine has a short C-terminal peptide conjugated to KLH with alum (Table 1). However, Th1 adjuvants regardless of the antigen can elicit a systemic inflammatory response that may act at the BBB to induce damaging immunological reactions affecting the CNS (34). Thus, it is very likely that CD4+ T-cells activated by Th1 adjuvants would secrete inflammatory cytokines that affect the CNS, regardless of A β lacking the T-cell epitopes. Another problem with truncated A β antigens is their induced antibody response, which targets monomers and plaques that are not effective therapeutic targets. Indeed, that many older people that have amyloid plaques are intellectually competent shows that plaque is not necessarily the cause of AD; i.e. it has been proposed that plaque is a way for the body to remove toxic A β . Yet, truncated A β peptides were used before the sequence-independent conformational epitopes in A β were identified using mAbs, since amyloids cannot be crystallized for analysis by X-ray crystallography (10,12). Nonetheless, if until recently the roles for monomeric and oligomeric A β in neurotoxicity were uncertain, the aducanumab study has showed that monomeric A β is not the right therapeutic target to prevent or treat AD.

Different from most N-truncated A β peptides, those having pyroglutamate as an amino terminal group at positions 3 or 11, A β N3 (pE) and A β N11 (pE) respectively, are potentially effective targets for immunotherapy (35). These modified peptides are more toxic than plain A β and their presence correlates with AD presence, i.e. they are absent from normal brains. Their value has been shown by passive immunotherapy, i.e. the treatment of transgenic mouse models for AD with mAbs directed against the terminal pyroglutamate reduces the A β plaque load and lower the levels of A β N3 (pE) and A β N11 (pE) forms (36). Hence, it is possible to consider these forms as likely vaccine antigen, perhaps in a polyvalent vaccine where a number of potentially relevant A β antigens are present.

Because of the past immunotherapy problems and the promising results with aducanumab and

solanezumab, is evident that an effective AD vaccine may require whole A β 42 to form the conformational epitope that is found in soluble A β oligomers and fibrils, but not monomers, as well as some truncated A β forms like A β N x (pE) (35). Studies with the canine model for AD (6), which is more similar to human AD than the transgenic mouse models, have shown that long-term vaccinations result in a progressive antibody response that drifts with time from recognizing linear A β epitopes to conformational ones (37). Thus, it is doubtful that an early anamnestic response will be helpful in AD vaccines, as the initial antibody response has no benefit in AD prevention and/or treatment; which explains why survivors vaccinated with AN1792, despite having for years high antibody titers and clearance of plaque, did not show any amelioration of the disease (33). Hence, development of an AD vaccine will require an unconventional approach.

Toward the rational design of an AD vaccine

Several lessons from the previous AD vaccine and aducanumab studies can be applied to this vaccine's development; particularly in view that methods from infectious disease vaccines may not be directly applicable to AD vaccines. Also, that this vaccine's requirements, like a need for whole A β and apparently tau with T and B-cell epitopes in a vaccine where a prerequisite is sole Th2 immunity, are rather conflicting, show the need for new strategies. While the Nabs and aducanumab studies have shown a requirement for the whole A β in order to present that conformational epitope crucial to induce a protective immunity, the studies with the AD canine model has revealed the complex chain of events leading to that response. Here we would address the roles of the vaccine's antigen and adjuvant, as well as strategies to elicit a sole Th2 immunity and prevent neuroinflammation.

A β 42 – the antigen That protective NAbs and aducanumab cannot recognize A β monomers indicates that there is no involvement of linear epitopes, usually identified by epitope mapping using short overlapping peptides of the antigen; which may be due to the amyloidogenic nature of this protein (12). Amyloids form fibrils, where the initial event is protein misfolding; these aberrant forms can start extensive oligomers and fibrils, which are associated with the amyloids' toxicity. Indeed, monomeric A β in aqueous solutions form aggregates with an intermolecular hydrogen bonded structural motif shared by pathogenic amyloids, which as indicated

before is sequence independent (38); an epitope(s) that presumably is recognized by aducanumab and Nabs. Although preparations of A β 42 show a wide variety of oligomers, the canine vaccine studies have shown that these preparations can be suitable vaccine antigens. Yet, it is possible that the production of stable oligomers with well conserved structures may be attained by intermolecular cross-linking and other alternative methods discussed later. Thus, the issue is how to use an antigen with the T and B-cell epitopes needed to form the conformational epitope that would stimulate production of protective antibodies, in a vaccine where Th1 immunity cannot be tolerated? The pragmatic approach to induce safely such immune response would be by using Th2 adjuvants; a challenge as most adjuvants induces Th1 with Th2 and/or Th17 immunities.

A viable alternative to the A β 42 antigen would be the use of peptide analogs that mimic the generic epitope(s) found in oligomeric and fibrillar A β (39); analogs that should be more stable and reproducible than A β preparations. A disadvantage of these antigens could be the induction of a narrower immune response against generic epitopes than that induced by whole A β ; a possibility that needs to be tested using different mAbs that recognize the natural conformational epitope(s). Clearly, the main advantage of these peptide analogs will be reproducibility, as it is important to induce early the correct immune response. Yet, the canine studies have shown that after prolonged immunizations, the immune system develops that protective immune response, even when using heterogeneous A β preparations (40). Another issue frequently overlooked, is the role of anti-A β IgMs that catalytically destroy this protein outside the CNS; but, different from Nabs and aducanumab, the IgMs recognize epitopes formed by specific A β amino acid sequences (21,22). Thus, IgM production will require vaccination with whole A β . Perhaps, an option would be a polyvalent A β vaccine having the antigens needed to stimulate both types of antibody response. Yet, regardless of the antigens, the immunity should be Th2, which would require new adjuvants.

Th2 adjuvants or immune modulators It is evident that the AN1792 vaccine damaging effects were caused by the adjuvant QS-21; a glycoside isolated from *Quillaja saponaria* Molina, a tree native to Peru and Chile. QS-21 is one of the few Th1 adjuvants that work without involvement of innate immunity receptors and it acts on T-cells and antigen presenting

cells, i.e. macrophages and dendritic cells (DC), inducing the production of CD4+ Th1 and CTLs (32). As discussed, the reportedly safe Th2 response induced by AN1792 during phase 1, points to problems with the original vaccine, i.e. de-acylation of QS-21, a chemical change that shifts the response from Th1 to Th2 immunity (41); a situation difficult to determine without cytokine analysis.

Adjuvants are usually recognized by the immune system as a signal that the body is under attack by pathogens, which trigger a Th1 immunoresponse. A fitting response against pathogens, but not for self-antigens such as A β , as an inflammatory response will cause organ damage. As adjuvants induce a systemic and not just a local immune response to fight infections through the body, it is unlikely that deleting an antigen's T-cell epitopes will avert that response. An effect of a systemic Th1 immunity is that the inflammatory cytokines might activate the BBB endothelial cells to secrete inflammatory mediators into the brain, initiating or aggravating inflammation, which is an undesirable situation in AD (42). Yet, while Th1 adjuvants are not acceptable in AD vaccines, because of the immune decline associated with aging, some kind of adjuvant would be needed to induce and maintain an effective Th2 immune response in the elderly.

Th2 anti-inflammatory adjuvants are rare and their origins seem to coincide with that of humoral immunity well over 200 million years ago. In fact, Th2 adjuvants are related to products made by parasitic helminths, which by inhibiting the host's inflammatory response and boosting the milder humoral one, assure their survival and long-term coexistence with the host (43). Interestingly, these compounds inhibit but do not eliminate the pro-inflammatory Th1 immunity, needed for protection against pathogens and incipient tumors. Helminths produce two types of Th2 immune modulators, one made of proteins and lipids that have phosphorylcholine (PC) and another that include various fucosylated glycans. Presently there is an experimental AD vaccine with PC as a Th2 adjuvant, but as PC derivatives are water insoluble this vaccine requires liposomes (42), which may limit its accessibility in parts of the world. Also, PC may induce under some conditions Th17 immunity that is linked to inflammation in autoimmune diseases; in contrast, fucosylated glycans are water soluble and easy to formulate. These glycans work by binding to DC-SIGN, a DC's C-type lectin, biasing DCs toward Th2 immunity while inhibiting the inflammatory Th1

immunoresponse (45). Thus, fucosylated glycans with A β 42 should elicit a Th2 antibody response and systemic anti-inflammatory immunity. Yet, their synthesis is costly.

By serendipity, the de-acylated derivative of QS-21 named QT-0101 is an effective Th2 adjuvant that different from QS-21 is stable and significantly less toxic. Its mechanism of action may be explained by the fact that de-acylation of QS-21 frees its single fucosyl residue, which presumably becomes available to bind to DC-SIGN and bias DCs toward Th2 immunity. Like the helminths' fucosylated glycans, it does not abrogate Th1 immunity, but inhibits it in a reversible manner (46). That QT-0101 facilitates the passage of proteins across mucosae would allow nasal delivery while avoiding intramuscular injections, as injections are hard on the elderly that have less muscle mass than younger people. This situation becomes acute with the long term immunizations, needed because of the immune decline associated with aging.

Since the neurological changes, decline in memory and immunological response in aging dogs, parallel those seen in AD, the long-term vaccination studies with the canine model using A β 42 are relevant to the development of a human vaccine. The canine vaccine used alum, a safe but weak Th2 adjuvant (40), as shown by the influenza vaccine that is highly effective in the young, i.e. around 80 to 90 percent protection, but it has a low efficacy in the elderly, i.e. as low as 30 percent. Moreover, alum does not induce a systemic anti-inflammatory response that would be of benefit in AD. Another problem associated with aging is immunosenescence, where T cells and DCs lose receptors and ligands that are needed for T cell activation and prevent anergy. Thus, an AD vaccine would benefit from an adjuvant like QT-0101, which in addition to eliciting a strong systemic Th2 immunity, may prevent T-cell anergy by delivering via its aldehyde group the co-stimulatory signal needed for T-cell activation. Nonetheless, the prolonged vaccination of dogs with A β 42 plus alum resulted in an antibody response that shifted from recognizing the monomeric A β N-terminal region to one that recognized a sequence independent conformational epitope (37). In fact, the late antibody response is similar to that found in Nabs and probably in aducanumab. Yet, there was no cognitive improvement in the vaccinated as compared to non-vaccinated dogs.

While many reasons may exist to account for the different results between the vaccinated dogs

and aducanumab studies, a conspicuous one is the amount of antibodies found in vaccinated dogs versus that used in the mAb study. Vaccinated dogs had IgG levels of around 37 μ g/mL of plasma (37), while a single aducanumab treatment delivers to the patient enough mAb to reach a level of 257 μ g/mL of plasma, or 10 mg/kg (47), i.e. 7 times higher. Another factor would be the antibody's affinity for its epitope, i.e. aducanumab was chosen due to its high affinity; while there is no information about the avidity of the dog antibodies, it is unlikely that it would be high, as alum does not induce an effective antibody affinity maturation process. Therefore, that the different results may be due to the antibody concentrations and their avidity raises the possibility that a vaccine inducing high levels of high avidity antibodies will show beneficial effects (48). Thus, a vaccine should have the right adjuvant to induce Th2 anti-inflammatory response with production of antibodies with a high avidity for the antigen. This situation suggests that vaccines may be more effectively used in a preventive mode in the younger immune competent population, rather than as therapeutic agents in the aged population suffering of immunosenescence. It is quite possible in view of the new developments and lessons from the past, that there would be an end to the stream of disappointments in AD drug-development (49,50).

CONCLUSIONS

The promising clinical studies with the mAb aducanumab showing that this drug reduces amyloid plaques and improves cognitive functions, strengthen the immunotherapeutic approach, while confirming the key role of A β in AD pathology and existence of a natural protective immunity against A β toxic forms. These findings also support the use of vaccines to prevent and/or treat AD, an approach that has yielded only failures. Evidently, an AD vaccine would need A β 42 as an antigen to allow formation of the conformation-dependent epitope, like that recognized by aducanumab. Indeed, these results were confirmed in the canine model, which showed that upon vaccination, the antibody response shifted from linear epitopes to final conformational epitopes; but, there was no improvement of the cognitive functions, a result that may be due to the lower antibody concentration compared with that of aducanumab. Thus, a vaccine should have besides A β 42 and potentially tau protein, a strong sole Th2 immunity adjuvant to induce an elevated production of antibodies with high affinity for the antigen, a response that alum may not deliver. Novel adjuvants based on PC and fucosylated

compounds could fill that need; especially QT-0101, a fucosyl glycoside with anti-inflammatory properties, which may also ameliorate T-cell anergy that is quite common in the aging population and responsible for a poor immune response.

Hence an effective AD vaccine should have A β 42, which apparently is processed by the immune system in various ways, synthetic analogs of that generic epitope (51), plus the N-truncated A β forms with pyroglutamic acid as the terminal residue, which are found only with AD and clearly have a role in the disease pathology. Due to the close relation between A β and tau in this disease progression, tau may be considered as another antigen. Thus, the vaccine should be able to elicit immune responses against different but relevant antigens, i.e. the vaccine would be a polyvalent vaccine with one caveat, because of its various self-antigens having all of their T-cell epitopes, the only safe and apparently required immune response would be a sole Th2 and preferentially if the Th1 immunity is inhibited, but not abrogated. This way the vaccine will induce a Th2 immune response against the antigens and a concomitant systemic anti-inflammatory immunity, which will be beneficial in ameliorating the inflammation associated with aging. It is expected that the vaccine would perform better in the younger population, i.e. below 65 years of age, than in the older one with more than 70 years of age; hence, as most vaccines it would be more effective when used in a preventive rather than therapeutic mode. Considering what it is known about the immunopharmacology of adjuvants, it is evident that the use of compounds that under any circumstances induce Th1 or Th17 immunities, would result in damaging side effects.

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