Title: VACCINE FORMULATIONS THAT INDUCE A TH2 IMMUNE RESPONSE

Abstract: The present invention relates to compositions and methods for the prevention and treatment of neurodegenerative diseases, such as Alzheimer's disease, that are caused by misfolding, aggregating proteins. The compositions and methods of the present invention comprise a vaccine formulation comprising an antigen selected from the group consisting of i) amyloid-β or a peptide that has in its amino acid sequence part of the amyloid-β amino acid sequence, ii) phosphorylated tau protein or one of its phosphorylated peptides, or iii) a combination of antigens derived from groups i) and ii) and that are formulated with a non-acylated or deacylated, natural or synthetic, bidesmosic triterpene glycoside carrying an aldehyde or ketone group, which acts as an adjuvant or immune agonist. These vaccine formulations are capable of stimulating a Th2 immunity or antibody response against antigens such as amyloid-β and tau derived antigens, but not a Th1 immune response.

Fig. 1
Vaccine Formulations that Induce a TH2 Immune Response

FIELD OF THE INVENTION

[0001] The present invention relates to immune modulatory formulations or vaccines that induce a strong Th2 immune response with production of antibodies against the proteins β-amyloid (Aβ), hyperphosphorylated tau protein and/or its peptides, peptide analogs and peptide conjugates, alone or in combinations, but do not induce a Th1 immune response. In particular, this invention pertains to vaccines containing non-acylated bidesmosidic aldehyde-carrying triterpene glycosides as adjuvants or immune agonists, that stimulate Th2 immunity with antibody production, but do not elicit Th1 inflammatory immune response with or without the production of cytotoxic T lymphocytes (CTLs), and that are useful in the treatment and/or prevention of Alzheimer's disease and other neurodegenerative diseases that are proteinopathies.

BACKGROUND OF THE INVENTION

Alzheimer's Disease, β-amyloid Protein and tau Protein

[0002] Neurodegenerative diseases, although caused by a variety of factors, share a certain common characteristic. In effect, many of these illnesses are proteinopathies or diseases caused by misfolded, aggregating proteins (Southwell AL et al. Rev Neurosci. 21:273-287, 2010). One such degenerative disorder is Alzheimer's disease (AD), a progressive neurodegenerative disease that affects largely the elderly population leading to dementia; while by age 65 the AD prevalence in this population is 7-10%, it increases severely to about 40% in the population over 80 years of age (Sisodia SS. J Clin Invest. 104:1169-1170, 1999; Fu, HJ et al. CNS Neurol Disord Drug Targets. 9:197-206, 2010).

[0003] The significance of this disease is highlighted by the World Health Organization's prediction that by the year 2050, AD would be the world's leading cause of death. A characteristic of AD is the deposition of the protein Aβ into clumps called oligomers on neural cells to form extracellular neuritic plaques, which seem to play a crucial role in its pathology (Crews L et al. Hum Mol Genet. 19:R12-R20, 2010; Gandy S. J Clin Invest. 115:1 121-1 129, 2005; Neniskyte U. et al. J Biol Chem.
In effect, the main neuropathological symptoms of AD associated with a progressive cognitive decline are the extracellular accumulation of Aβ plaques, the intracellular formation of neurofibrillary-like structures (NFTs) composed of paired helical filaments with phosphorylated tau proteins (Selkoe DJ. *Ann N.Y. Acad Sci.* 924:17-25, 2000), and the neuronal and synaptic loss. Most Aβ consist of two isoforms, a main peptide called Aβ40 that contains amino acid residues from 1 to 40 and a minor one that is less than 5% of the Aβ, which has 2 extra amino acids, ending at residue 42 instead of 40, and which is named "long Aβ" or Aβ42 (Naslund J. *et al.* *Proc Nat Acad Sci USA.* 91:8378-8382, 1994). While both Aβ isoforms have a propensity to form β-sheets, because the long Aβ isoform, Aβ42, has more aggregability than Aβ40, it is believed that it starts the process leading to the formation of oligomers, fibrils and plaques (Gravina SA. *et al.* *J Biol Chem.* 1270:7013-7016, 1995; Younkin SG. *Ann Neurol.* 37:287-288, 1995). Indeed, it has been proposed that these aggregated states of Aβ are those with the most potent neurotoxicity and responsible for AD. Support for this proposal is provided by the prion diseases, e.g. Creutzfeldt-Jakob disease, where the misfolding of amyloidogenic peptides leads to neurotoxicity, but without plaque formation (Gandy S. *J Clin Invest.* 115:1 121-1129, 2005).

Although it is unclear if the accumulation of Aβ is either the decisive neurotoxic end-product or a by-product of an independent metabolic lesion that happens to be neurotoxic, the removal of Aβ plaques by certain immunological methods have shown a recovery of cognitive functions in a transgenic mouse model for AD (Schenk D. *et al.* *Curr Opin Immunol.* 16:599-606, 2004; Wilcock DM. *et al.* *J Alzheimer's Dis.* 15:555-569, 2008). After these results were reported using a vaccine having Aβ42 and the adjuvant QS-21, formulation AN-1792, (Schenk D. *et al.* *Nature* 400:173-177, 1999) (US 6,905,686 Bl), several groups confirmed and extended those studies concerning the effects of active immune therapy in preventing deposition of Aβ and improving the cognitive functions in a mouse transgenic model (see Tabira T. *Tohoku JExp Med.* 220:95-106, 2010; Wilcock DM. *et al.* *ASNNeurol.* 3:249-258, 2011; Chackerian B. *Hum Vaccin.* 6:926-230, 2010; Wiessner C. *et al.* *J Neurosci.* 31:9323-9331, 2011).

While the results from a transgenic mouse model were encouraging from both the therapeutic and safety aspects, clinical studies using AN-1792 were terminated due to the meningoencephalitis triggered by the vaccine in 6% of the patients during a
Phase II clinical trial. Evidently, this side effect was the result of a cell mediated inflammatory response caused by the combination of certain epitopes from the antigen Aβ42 (Gelinas DS et al. Proc Nat Acad Sci USA. 101:14657-14662, 2004) and the QS-21 adjuvant, which elicits Th1 immunity with production of cytotoxic lymphocytes or CTLs (Kensil CR et al. Dev Biol Stand. 92:41-47, 1998). In fact, autopsy of a patient immunized with AN-1792 showed the presence of T-lymphocyte meningoencephalitis and infiltration of cerebral white matter by macrophages (Nicoll JA et al. Nature Med. 9:448-452, 2003).

Nonetheless, a subsequent evaluation of the patients enrolled in that study, showed that those that developed antibodies against Aβ42 may have benefited cognitively from the vaccine (Gilman S et al. Neurology 64:1553-1562, 2005). While autopsies of some of the study participants showed a decrease in Aβ plaques, it has been difficult to establish a correlation between this decrease and improvement of cognitive functions (Holmes C. et al. Lancet 372:216-223, 2008). A more controlled study to minimize contamination by plaque Aβ has shown a strong correlation between the levels of Aβ and cognitive status (Naslund J. et al. JAMA 83:1571-1577, 2000). These results are more in line with those reported for the transgenic mouse model. An outcome of these reported studies has been a surge of activity trying to develop active and passive immunization methods targeting Aβ, in order to prevent or reduce the plaques load in the brain.

Another protein closely associated with AD is the tau protein, a microtubule-associated protein that stabilizes the microtubule structure that in the adult brain is present in six isoforms, which are characterized by unusually high ratios of hydrophilic amino acids and proline. These six isoforms differ according to their content of i) tubulin binding domain repeats, i.e. 3 or 4 repeats of 31 to 32 amino acids in the C-terminal part of tau, and ii) number of specific inserts of 29 amino acids each, in the N-terminal region of the protein (Kolarova M. et al. Int J Alzheimer's Dis. 2012:732526, 2012). Tau isoforms vary in size from 352 to 442 amino acid residues. Results of this amino acid composition are its high solubility and the lack of a strict secondary structure with the peptide chain being in a random coil state (Huang HC et al. J Alzheimer's Dis. 16:15-27, 2009). Most tau isomers are found at axons, with only a few located at neuronal cytoplasm and dendritic cells.

Tau's functionality is tightly regulated by its degree of phosphorylation, with too much or too little phosphorylation altering its conformation and affecting its
biological functions (Ubhi K et al. Exp Neurol. 230:157-161, 2011). In pathological conditions tau is hyperphosphorylated, which results in a decrease of its binding to microtubules and the presence of isolated tau in the neurons; the isolated tau being susceptible to form paired helical filaments when 8 to 10 of the at least 30 available sites are phosphorylated. However, it has been proposed that to cause pathological changes, tau hyperphosphorylation must be at specific sites, but not others (Avila J. Front Neurosci. 4:49, 2010). Different studies indicate that in tau paired helical filaments the dominant structure is β-sheet, a structure that can contribute to conformational changes in that protein and its aggregation, which play a key role in its neuronal toxicity by forming intracellular NFTs (Kolarova M. et al. Int J Alzheimers Dis. 2012:732526, 2012). These neurodegenerative disorders caused by the tau protein's conformational alterations are known as tauopathies, with AD being the most prevalent.

A body of evidence indicates that formation of pathological tau conformers seems to occur with or after the initiation of Aβ aggregation and induction of neurotoxicity. Support for this concept is given by the fact that Aβ dimers isolated from the brains of late-onset AD patients and at subnanomolar concentrations are enough to induce hyperphosphorylation of tau at epitopes that are AD-relevant, initiating a disruption of the microtubule cytoskeleton and causing neuritic damage (Ming J et al. Proc Nat Acad Sci USA. 108:5819-5824, 2011).

However, studies with knockout and transgenic mice seem to indicate that the induction of AD may be the result of a combination of factors, which include conformational defects in Aβ, tau and perhaps some other proteins. In effect, it has been proposed that accumulation of Aβ in neurons may induce tau phosphorylation by alternative pathways, in agreement with reports showing that Aβ dimers can induce hyperphosphorylation. It has been also shown in transfected primary neurons that expression of truncated tau resulted in mitochondrial fragmentation in these cells, which was aggravated by exposure of the cells to sub-lethal concentrations of Aβ; results that indicate some kind of cooperation between those proteins (Quintani-Ua RA et al. Neurobiol Aging. 33(3):619.e25-35, 2012).

Therefore, it is becoming more accepted that the toxicity of Aβ is tau dependent. For instance, it has been shown that in transgenic mice expressing a truncated form of tau (Atau) or with an absence of tau (tau<sup>-/-</sup>) and Aβ-forming, memory deficits are prevented and have an improved survival rate as compared to
mice producing the normal tau protein (Ittner LM et al. Cell 142:387-397, 2010). Results that indicate that tau confers at least some of Aβ toxic properties. Thus, it now more accepted that AD may be a result of damage caused by cooperative effects between Aβ and tau; while, these 2 proteins seem to be crucial for starting AD, it is possible that other proteins may also play a role.

Similar to Aβ, because the tau protein’s pathological effects seem to be the result of conformational changes, it is a suitable candidate for passive and/or active immune therapy, where the defective isoforms are removed by interactions with specific antibodies, this way reducing the extracellular Aβ neuritic plaques and intracellular tau NFTs. Indeed, it has been shown that immunization of a transgenic mouse for a tau mutant that develops early NFTs, with the peptide Tau379-408[P-Ser,9,6,404] plus aluminum phosphate as an adjuvant, produced tau-antibodies that recognized the intracellular NFTs, reducing the aggregated tau in the brain and slowing the progression of the tangle-related behavioral phenotype (Asuni AA et al. J Neurosci. 27:91 15-9129, 2007; Sigurdsson EM. J Alzheimer’s Dis. 15:1 57-168, 2008; US 8,012,936 B2; US 2002/0197258 Al). The fact that these tau-antibodies are attached to NFTs, shows that in addition to passing across the blood-brain barrier (BBB), they can also penetrate the cell; yet, these antibodies could not cross the BBB in wild type mice, indicating some deterioration of this barrier caused by neurodegenerative diseases. Similar results have been reported in transgenic mice expressing Tau22 and that were immunized against the pathological epitope phosphor-Ser422 combined with CFA (Troquier L. et al. Curr Alzheimer Res. 9:397-405, 2012). The results indicate that active immunization resulted in Tau clearance and the improvement of cognitive deficits caused by tau pathology.

Additional proof of the potential therapeutic efficacy of tau vaccines comes from vaccination with the peptide Tau379-408[P-Ser,9,6,404] of transgenic mice htau/PSI, model that has an early onset and more aggressive progression of tau pathology than the htau model (Boutajangout A. et al. J Neurosci. 30:16559-16566, 2010). In this model, the results show that vaccination totally prevents severe cognitive impairment.

An explanation for the antibody-mediated clearance of tau aggregates has been obtained using an ex-vivo brain slice model. FITC labeled anti-tau antibodies show that i) the antibodies were localize on the phosphorylated tau, ii) are co-localized with markers of the endosomal/lysosomal pathway and iii) the tau-antibody complex were
found together in an enriched lysosome fraction, indicating that antibody-mediated clearance of intracellular tau aggregates seems to occur via the lysosomal pathway (Krishnamurthy PK et al. Front Psychiatry 2011:2:59). Similar results to those obtained by vaccination have been attained by the administration of monoclonal antibodies against tau epitopes that are relevant for its pathogenic properties. Administration of antibodies against tau pathological forms to transgenic mice showing a progressive tauopathy, resulted in a reduction of tau pathology and prevention of tau intracellular buildup (Chai X et al. J Biol Chem. 286:34457-34467, 2011). Another group reported similar results using a mAb that targets the pathological phospho-tau protein, a treatment that resulted in decrease of both tau pathology and functional impairments in a transgenic mouse model (Boutajangout A. et al. J Neurochem. 118:658-667, 2011).

Although the immunotherapy approach for treatment of tauopathies has been focused on the hyperphosphorylated tau, some groups have proposed that tau oligomers, i.e. aggregates between the size of monomers and NFTs, should be the target of immunotherapy. The bases for this proposition is the evidence that neuronal loss precedes NFTs formation and that tau oligomers can cause neurodegeneration and memory impairment in the absence of Aβ (Kayed R. Hum Vaccin. 6:931-935, 2010). Yet, that immunization with the full-length tau resulted in a severe autoimmune reaction, rules out the use of soluble tau as an immunogen and stresses the need for identification of epitopes highly specific for early tau damage. Hence, the available data shows that immunotherapy producing antibodies that target different tau protein structures is a viable option in AD treatment, possible in conjunction with immunotherapy of Aβ because of the apparent synergism between Aβ and tau pathological effects.

*The Immune System*

Vertebrates exhibit both non-specific immunity (also referred to as innate immunity) and specific immunity (also referred to as acquired or adaptive immunity). Innate immunity ligands, which are pathogens' products, upon recognition by receptors encoded in the animal's genome trigger a defensive response involving cytokine production, activation of complement and natural killer cells and the identification and removal of foreign substances by specialized white blood cells; i.e.
these ligands are exogenous adjuvants or immune agonists (Akira S et al. Cell 124:793-801, 2006).

Innate immunity may also start the specific humoral and cell-mediated immunities, carried out by B and T lymphocytes together with other cells. B cells participate in humoral immunity or Th2 immunity when activated to produce antibodies by antigen presenting cells (APCs) and CD4+ Th2 helper T cells (Iwasaki A et al. Science 327:291-295). The Th2 immunity is characterized by the production of non-cytolytic antibodies and anti-inflammatory cytokines. Cell-mediated immunity or Th1 immunity, involves cytokine production by Th1 helper T cells, activation of macrophages and antigen-specific CD8+ cytotoxic T-cells (CTLs) to destroy pathogens. Activation of CD4+ and CD8+ T cells requires two signals: one derived from the T cell receptor (TCR) interaction with antigen-major histocompatibility (MHC) complexes on APCs, and the second a co-stimulatory signal delivered by CD80 or CD86 ligands (also known as B7-1 and, B7-2, respectively) on the APCs when binding to the CD28 surface receptor on T cells (Smith Garvin J et al. Annu Rev Immunol. 27:591-619, 2009). The result of a concerted stimulation with CD80 is Th1 immunity with the production of pro-inflammatory cytokines and CTLs that are crucial to destroy tumor and virally infected cells (Feili-Hariri M et al. J Leukoc Biol. 78:656-664, 2005).

Immunization with the AP42/QS-21 vaccine formulation of transgenic mice and patients in the Phase 1 clinical studies elicited a Th2 immune response, while the patients in the Phase 2 clinical studies exhibited a Th1 immune response. However, the vaccine formulation used in the Phase 2 clinical studies that elicited Th1 immunity was not identical to the one used either in the mouse model or the Phase 1 clinical studies, which elicits Th2 immunity. The difference between both formulations was that the one that elicited Th1 immunity contained a non-ionic detergent, polysorbate-80, presumably to aid the manufacturing process and stability of Aβ42; in contrast, the formulation that elicited a Th2 immunity was lacking the detergent (Schenk D. et al. Curr Opin Immunol. 16:599-606, 2004).

Thus, as a result of the active immune therapy results, a great deal of activity has been devoted to design new peptide antigens related to Aβ and denominated here "Aβ-derived peptides" to maximize the production of anti-Aβ antibodies, while restricting the Th1 immune response. For instance, shorter Aβ-derived peptides lacking T-cell epitopes have been used in vaccines that in the transgenic mouse model

A different tactic to induce only a Th2 immune response has been the use of mutated Aβ (Cao C. et al. BMC Neurosci. 9:25, 2008) and of cyclic peptides from Aβ (Hoogerhout P. et al. PLoS One. 6(4):el91 10, 2011). These and other Aβ-derived peptides have been described in the following patents and patent application US 6,787,637; US 6,861,057; US 7,067,133 B2; US 7,588,766; US 8,022,180 B2; US 8,034,348 B2; US 8,034,353 B2; US 2002/0094335 Al; EP 1420815 Bl; 2007/0135337 A2; US 2009/0202627 Al; US 2010/006201 I Al; US 201 I/0002949 Al; US 201 I/0182928 Al; US 201 I/0206706 Al; US 201 I/0206742; US 201 I/0262458 Al; US 2012/0052086 Al; 2012/0315321 Al). Other methods to elicit Th2 immunity against Aβ involve the use of DNA vaccines to express antigens similar to those indicated above (EP 2173375 Al; US 2012/0014987 Al), alone or in conjunction with a virus or a virus like particle (US 6,719,970; US 6,964,769; US 7,264,810; US 7,279,165; US 7,479,280; US 7,875,450; US 7, 8,318,687).

Similar to Aβ-based vaccines, tau-based vaccines apparently have the same restrictions about Th1 immunity. Administration to a transgenic mouse model for AD of lipopolysaccharide (LPS), a TLR4 ligand and known inducer of inflammation, resulted in activation of microglia and tau phosphorylation, but it did not affect Aβ (Kitazawa M et al. J NeuroSci. 25:8843-8853, 2005). LPS exacerbated tau pathology via the kinase cdk5. Thus, it is apparent that any immune response in the CNS must be confined to Th2 immunity producing antibodies only. Another report showing the damaging effects of an inflammatory response on the CNS, is one in which full length tau was administered with CFA, a powerful Th1 inflammatory adjuvant. Vaccinated non-transgenic mice developed autoimmunity and showed the histopathologic features typical of AD and tauopathies, stressing the dangers of using full length tau with presumably T and B epitopes and a Th1 adjuvant (Rosenmann H et al. Arch Neurol. 63:1459-1467, 2006). Because of these results, it is obvious that tau-based vaccines must be formulated with only tau's B epitopes and without a Th1 adjuvant, like lipid A or CpG, to avoid stimulation of a damaging Th1 inflammatory
response; situation that parallels that of the Aβ-based vaccines for the treatment or prevention of AD and that should be the norm for any vaccine that stimulates an immune response that acts on the CNS.

Due to the side-effects caused by a pro-inflammatory Th1 immune response, such as that elicited by QS-21 or lipid A, alternatives to stimulate only Th2 immunity to produce protective antibodies have been investigated, including other adjuvants, substitute administration modes, some delivery systems and different carriers.

One approach has been the use of Aβ40 without any adjuvant and administered intra-nasally, to produce antibodies against the amino acid sequence 1-15, recognized as the B-cell epitope. This immunization mode resulted in a Th2 immune response and reduced levels of Aβ plaques in transgenic mice (Town T. CNS Neurol Disord Drug Targets. 8:114-127, 2009).

Another group has used mutated Aβ peptides, but without an adjuvant to stimulate in mice Th2 immunity with antibody production, apparently with good results. Another method to avoid the inflammatory response has been the use of adjuvants that stimulate Th2 immunity with antibodies production. For instance, immunization of younger transgenic mice with Aβ42 without T-cell epitopes plus alum yielded an effective antibody response with reduction of Aβ burden but no cerebral microhemorrhages. Yet, that immune response did not occur in older mice, a deficient response that may be due to immunosenescence as well as the weak adjuvant used (Asuni AA et al. Eur J Neurosci. 24:2530-2542, 2006), a critical factor considering that AD affects predominantly the ageing population.

To boost the Th2 immune response stimulated by alum, the switching of alum to Quil A, a preparation of quillaja saponins that contains QS-21, has been tried (Ghochikyan A et al. Vaccine 24:2275-2282, 2006). According to these authors, changing from alum to Quil A increases the antibodies levels without changing the Th2 antibodies profile; yet, there is a significant reduction of the IgG1/IgG2a ratio, a strong indicator of a Th1 biased immunity. While the discrepancy about the antibodies' profile can be explained by the fact that Quil A and QS-21, stimulate both Th1 and Th2 immunity, a reduced IgG1/IgG2a ratio after treatment with Quil A, shows that IFN-γ, a Th1 cytokine, is being secreted (Finkelman FD et al. J Immunol. 140:1022-1027, 1988). Also, the large increase in IgG2b reported after the switch to Quil A, indicates secretion of IL-12, a Th1 driving cytokine (Germann T et al. Eur J Immunol. 25:823-829, 1995).
Although it has been presumed that a switch from alum to Quil A does not induce Th1 immunity while enhancing the production of anti-Aβ antibodies, and thus proposed as a safe and beneficial treatment for AD patients (see Fu HJ et al. CNS Neurol Disord Drug Targets, 9:197-206, 2010), it is possible that the change in immunity triggered by Quil A and leading to stimulation of Th1 immunity and down-regulation of Th2 immunity, may lead to undesirable autoimmune responses against the naturally occurring Aβ. Other strategies involve the use of Aβ peptides that are B cell epitopes and that are connected sequentially either to T cell epitope sequences unrelated to Aβ or are conjugated to a carrier protein that would then stimulate the T cells, this way avoiding an anti-Aβ inflammatory response (Agadjanyan MG et al. J Immunol. 174:1580-1586, 2005; Verdoliva A et al. Hum Vaccin. 6:936-947, 2010). Conjugates of the polysaccharide mannan, a Th2 adjuvant, and an Aβ-derived peptide, Aβ28, have been used to immunize APP transgenic mice; while the immunization produced antibodies that prevented Aβ-plaque deposition, it also increased the risk of microhemorrhages in the brains of vaccinated animals; apparently a result of the anti-Aβ antibodies stimulated by the mannan conjugate (Petrushina I et al. J Neuroinflammation 5:42, 2008) Several of these antigen constructs are the subject of issued patents or pending patent applications.

To prevent Th1 immunity while inducing an effective Th2 immune response with protective antibodies, Aβ-derived peptides that are B-cell epitopes have been expressed or covalently conjugated to virus like particles (VLP) derived from the E. coli phage Qβ, which provides a scaffold to link the peptides as well as T-helper cell epitopes (Wiessner C et al. J Neurosci. 31:9323-9331, 2011; Chackerian B et al. Vaccine 24:6321-6331, 2006; US 6,719,978 B2; US 6,964,769; US 7,264,810; US 7,279,165; US 7,320,793; US 7,371,572 B2; US 7,479,280 B2; US 7,494,656; US 7,875,450 B2; US 2004/0141984 Al; US 2009/0246215 Al; US 2013/001 1431 Al). The use of VLPs apparently facilitates the production of antibodies against tolerogens or self-antigens, such as Aβ.

An alternative strategy has been the use of retrovirus-like particles having only the gag and pol proteins from murine leukemia virus, but displaying Aβ-peptides, i.e. Aβ retroparticles, to stimulate Th2 immunity against Aβ (Back P et al. J Immunol. 182:7613-7624, 2009).

In all cases, the vaccines have been designed to stimulate production of Th2 immunity with anti-Aβ antibodies that reduce the Aβ load, while avoiding Th1
immune pro-inflammatory responses, either without adjuvants or with adjuvants that elicit only Th2 immunity. Hence, most of the attention has been focused on the antigen rather than the adjuvant component; evidently a result of the inflammatory response observed during the phase 2 clinical studies with the Aβ vaccine AN-1792 containing QS-21. Of significance is that the Th1 inflammatory response took place only after modification of the original vaccine formulation by adding the non-ionic detergent polysorbate-80, for use in the phase 2 clinical studies; a response that has been explained by the exposure of Aβ T-cell epitopes due to the detergent combined with the use of an effective Th1 adjuvant. However, it is most likely that the main target of the detergent action was the QS-21 adjuvant rather than the antigen, i.e. it has been shown that addition of non-ionic detergents to QS-21, Quil A and other q. saponin analogs, causes a large enhancement of their adjuvanticity, 10-fold or more.

Thus, it is feasible that an incipient immune response, result of a supposedly suboptimal formulation, was magnified by the large increase in the QS-21 adjuvant activity. It is dubious that low concentrations of the non-ionic detergent polysorbate-80, would have significant effects on the Aβ structure; i.e. oligomeric Aβ can only be dissociated by using high concentrations of formic acid, strong chaotropic agents, like Gu-HCl, or detergents as sodium dodecyl sulfate (SDS) under stringent conditions (Masters CL et al. Cold Spring Barb Perspect Med. 2:a006262, 2012). However, the use of Aβ-derived peptides lacking T-cell epitopes has confirmed that those epitopes, in combination with what it was apparently a "high dose" of QS-21, were responsible for the Th1 pro-inflammatory response.

Analogous to the Aβ vaccines constrains on stimulation of Th1 immunity, the tau vaccines that have shown beneficial results in transgenic mouse models for AD, are those with phosphorylated short tau peptides as immunogens, in some cases conjugated to carrier proteins. In contrast, tau vaccine formulations containing the full length tau plus a Th1 adjuvant, like LPS or CFA, stimulated a damaging inflammatory response. Yet, the alternative of using Aβ or tau-based vaccines without an effective adjuvant or one like alum, a Th2 adjuvant that stimulates IgG production but fails to stimulate an effective immune response in aged mice, presents a serious problem because in humans and some mammals, the target populations for AD or AD-like conditions are the aged ones. This situation stresses the need for effective
adjuvants in the AD vaccines to stimulate a beneficial Th2 immune response in the elderly while overcoming the effect of immune senescence and preventing tolerance.

[0032] Immune senescence or the deterioration of the immune system linked to aging, is one of the main challenges to the development of vaccines for the elderly (Gruver A et al. J Pathol. 211:144-156, 2007; Ron-Harel N et al. Trends Neurosci. 32:367-375, 2009; Ongradi J et al. Immun Ageing 7:7, 2010). Aging affects APCs, i.e. dendritic cells and macrophages, by reducing the expression of toll-like-receptors (TLRs) associated with innate immunity (Aspinall R et al. Immunity and Ageing 4:9, 2007), and the co-stimulatory ligands CD80 and CD86 associated with adaptive immunity.

[0033] Another significant aging-related change is the decrease in expression of the CD28 receptor for the B7 ligands in CD8+ and CD4+ T cells (Plackett TP et al. J Leukoc Biol. 76:291-299, 2004); these reduced expressions of the APCs' co-stimulatory ligands and T cells' CD28 receptor, are major factors contributing to the immunity decline in the elderly, which leads to tolerance. Immune senescence also occurs in the brain, causing the microglia, which are the macrophages equivalent (Gemechu JM et al. Front Cell Neurosci. 6:38. 2012; Wrona D. J Neuroimmunol. 172:38-28, 2006; Streit WJ et al. Aging Dis. 1:254-261, 2010), to function atypically and promote neurodegeneration (Luo X-G et al. Mol Neurodegener. 5:12, 2010). However, as the BBB acts as a filter that shields the brain from molecules and cells from the blood milieu, the brain is an immune privileged organ; thus, it is unlikely that Aβ or tau-derived antigens from vaccines would be processed by the brain's microglia. Hence, like other antigens, initial processing of tau or Aβ-derived antigens should take place at the APCs, with the subsequent secretion of Th1 or Th2 cytokines and interactions with T cells leading to T cell activation and production of CD4+ or CD8+ T cells, functions that can be modulated by different adjuvants (Marciani DJ. Drug Discov Today. 8:934-943, 2003). While there is evidence that in AD the peripheral T cells, CD4+ and CD8+ T cells, can enter the brain and infiltrate areas with Aβ-plaques, T cells may also exert their effects without entering the brain, via Th1 and/or Th2 cytokines, e.g. IFN-γ, TNF-a, IL-10, and other factors (Jozwik A et al. PLoS One 7(3):e33276, 2012; Fisher Y et al. PLoS One 5(5):e9830, 2010).

[0034] Thus, immune senescence may affect the immune system by blocking the production of cytokines by anergic peripheral T cells and imposing tolerance; a situation that may impact the production of protective antibodies. In fact, down
regulation of the co-stimulatory APC ligands, CD80 and CD86, and/or their T cell's receptor CD28, by eliminating the required co-stimulatory signal causes T cell anergy (Mondino A et al. *J Leukoc Biol.* 55:805-815, 1994; Su-Yi T et al. *Current Opin Cell Biol.* 14:575-580, 2002). Because this condition may be common in the elderly AD population, it is unlikely that vaccines without an effective adjuvant or immune agonist would stimulate a useful protective antibody response against Aβ or tau protein in many of those patients. Essentially, the adjuvant(s) required for an AD vaccine would need to deliver an alternative T cell co-stimulatory signal to replace the one delivered by the down-regulated CD80/86 ligands to allow T cell activation and elicit only a Th2 immune response with production of protective antibodies, a task that adjuvants like IFA, alum and many others cannot deliver.

*Saponin adjuvants*

[0035] The only well-characterized exogenous immune agonists or adjuvants that can deliver an alternative co-stimulatory signal are Quil A and its components, such as QS-21, QS-18, QS-17 and QS-7. Structurally, these quillaja saponins are glycosides that have as an aglycone a lipophilic triterpene, which is linked to two oligosaccharide chains bound at positions C-3 and C-28. Because of the presence of both hydrophilic and lipophilic structures, saponins have an amphipathic character with detergent-like properties. It is due to these properties and that the triterpene can insert into the cholesterol of the membrane's lipid bilayer, that quillaja saponins have a translocating capacity, i.e. allow the passage of proteins across the cellular membranes, such as the cell and endosomal membranes, directly into the cytosol.

[0036] Most of these saponins, with the exception of QS-7 that is acetylated, have their fucosyl residue acylated with an acyl-acyloyl moiety composed of two 3,5-dihydroxy-6-methyloctanoic acid residues linked in tandem (R Higuchi et al. *Phytochemistry* 26:229-235, 1987; *ibid*, 27:1 165-1 168, 1988) and de-acylation occurs under mild conditions, i.e. above pH 6 and at room temperature (RT), a situation that apparently results in a loss of these saponins' capacity to insert and pass across the cell's membrane, to deliver an antigen directly into the APC's cytosol to trigger CTL production. Structure/function studies have shown that the aldehyde group is essential to stimulate immunity and that its modification results in a loss of the immune stimulatory capacity (Soltysik S et al. *Vaccine* 13:1403-1410, 1995). In effect, synthetic mannosylated triterpene glycosides based on oleanolic and glycyrrzic
acids that lack an aldehyde group, do not show immunological activity (Daines AM et al. Bioorg Med Chem. 17:5207-5218, 2009), a result of the lack in those glycosides of a group capable of providing a co-stimulatory signal to the T cells. In fact, from a variety of triterpene saponins, only those carrying an aldehyde group, i.e. *Quillaja* saponins, *Gypsophila* saponins and *Saponaria* saponins, have adjuvant activity (Bomford R et al. Vaccine 10:572-577, 1992). Furthermore, it is evident that the lipophilic acyl moiety of quillaja saponins is also required for stimulation of Th1 immunity with CTL production, as de-acylated quillaja saponins or DS-QS (Fig. 1) stimulate Th2 immunity without CTLs (Marciani DJ et al. Int. Immunopharmacol. 2001; 1:813-818; Liu G et al. Vaccine 20:2808-2815, 2002).

Hence, it is apparent that an acyclic alkyl chain, bound to the triterpene is essential to allow its passage, together with the antigen, across the cell and endosomal membranes for processing by the endogenous pathway, a requirement to yield Th1 immunity with CTL production (Marciani DJ et al. Vaccine 21:3961-3971, 2003). Of relevance is that while a small co-stimulatory Schiff-base-forming drug, 4-(2-formyl-3-hydroxyphenoxymethyl benzoic acid) or tucaresol (Rhodes J et al. Nature 377:71-75, 1995; US 5,958,980), favors a Th1-biased immunity with a Th1-type cytokine profile and concomitant CTL production, DS-QS and other non-acylated aldehyde carrying saponins stimulate Th2 immunity. Important for an AD vaccine is that while DS-QS stimulates a Th2 immunity, because of its aldehyde group still has the capacity to deliver to T cells the co-stimulatory signal needed for their activation and prevent anergy (Hall SR et al. Immunology 104:50-57, 2001).

Moreover, the Th2 immune response seems to be independent of the presence or absence of T cell epitopes in the antigen, i.e. while mice immunized with ovalbumin (OVA) plus QS-21 or Quil A developed a Th1 immune response with the concomitant production of OVA-specific CTLs, mice immunized with OVA and DS-QS develop a Th2 immune response without either CTL production (Marciani DJ et al. Int. Immunopharmacol. 2001; 1:813-818) or secretion of IFN-γ. Though OVA has a peptide sequence, OVA$_{323-339}$, which has multiple overlapping T cell epitopes and MHC-binding registers (Robertson JM et al. J Immunol. 164:4607-4712, 2000), immunization with DS-QS failed to elicit Th1 immunity and CTL production; in contrast, immunization with the acylated glycosides Quil A or QS-21 plus OVA, stimulated both a strong Th1 immunity and OVA-specific CTL production.
These results show that DS-QS and similar natural or synthetic non-acylated saponins modulate the immune response against OVA in a manner independent of the presence of T cell epitopes in the antigen. In fact, saponins that have small acyl groups like acetyl, e.g. QS-7 (US 6,231,859 Bl) (Fig. 2), behave to some degree similar to DS-QS. However, QS-7 at higher doses stimulates the production of antigen-specific CTLs (US 6,231,859 Bl; 6,524,584 B2), indicating that some degree of acylation is required for these glycosides to stimulate Th1 immunity.

SUMMARY OF THE INVENTION

The present invention is directed to vaccine formulations for the prevention or treatment of Alzheimer's disease, a disease of the elderly characterized by the deposition of Aβ plaques on neural cells in the brain and intracellular accumulation of hyperphosphorylated tau in the form of NFTs in the same cells, leading to their death and other neurodegenerative diseases. Some antibodies produced against the Aβ and tau proteins after passing across the BBB can break and interfere with the formation of plaques or NFTs respectively, or alternatively, they can act as a peripheral sink outside the CNS, clearing the circulating Aβ, this way removing the burden from neural cells. Thus, an Aβ or tau-specific antibody response or Th2 immune response, may be beneficial to prevent or stop the disease; however, an inflammatory immune response or Th1 immune response can be damaging to the neural cells. Currently, several vaccines have been proposed in which Aβ-derived antigens having only B-cell epitopes are formulated without or with adjuvants that stimulate only Th2 immunity, such as alum. A similar approach has been also use with some tau-derived peptide vaccines in transgenic mouse models for AD. A major problem with that approach is that AD occurs largely in the elderly population that due to the aging process suffers of immune senescence, a condition characterized by the loss of receptors and ligands on APCs and T cells, which lead to T cell anergy or tolerance. Hence, an effective Aβ or tau-based vaccine should besides stimulating Th2 immunity, ameliorate the effects of immune senescence; a condition that antigen-alone vaccines or formulated with currently available Th2 adjuvants, or DNA vaccines expressing the antigen alone or as part of a larger protein or a VLP, is doubtful that they would be able to overcome.

The present invention ameliorates those deficiencies due to immune senescence by in addition to stimulating only Th2 immunity with production of anti-
Aβ or anti-tau antibodies, even in the presence of T cell epitopes, delivers an alternative co-stimulatory signal needed for T cell activation; an objective that is achieved by formulating the Aβ or tau-derived antigens with one or more non-acylated bidesmosidic triterpene glycosides or saponins that carry an aldehyde or ketone group. These glycosides that act as adjuvants or immune agonists, in addition to providing a T cell co-stimulatory signal via their aldehyde or ketone group, would allow the antigen processing required for stimulation of Th2 immunity with production of anti-Aβ or anti-tau antibodies, but, without the stimulation of Th1 immunity.

In one embodiment, the present invention can be used as a preventive vaccine to elicit the production of anti-Aβ or anti-tau-antibodies, or both types of antibodies, which would later either avert or slow down the formation of Aβ plaques and NFTs in the brain's cells, thus preventing or delaying the onset of AD while reducing its severity. In another embodiment, the present invention can be used as therapeutic vaccine to elicit the production of antibodies against Aβ or tau, or both antigens, that would bind and disrupt the Aβ deposited as plaques in the brain or the intracellularly NFTs in the neurons, improving the prognosis of the disease.

**BRIEF DESCRIPTION OF THE DRA WINGS**

Fig. 1 shows the structure of DS-QS having a trisaccharide at C-3 and where the acyl-acyloyl group has been removed from the fucosyl residue at C-28 by mild alkaline hydrolysis.

Fig. 2 shows the structure of QS-7 that has an acetyl group at the fucosyl residue bound at C-28 of the triterpene.

Fig. 3 shows the structure of a non-acylated bidesmosidic saponin from *Gypsophila paniculata* having gypsoxygenin as the aglycone, with a tetrasaccharide at C-3 and a pentasaccharide at C-28.

Fig. 4 shows the effects of increasing DS-QS doses in the administered vaccine formulations on the production of the different anti-OVA IgG isotypes, IgGl, IgG2a and IgG2b, as compared to the effects of Quil A, 10 µg, and alum, 300 µg.

Fig. 5 (A) shows the effects of increasing DS-QS doses in the vaccine formulations on the ratio between the anti-OVA IgGl and IgG2a end point titers (IgGl/IgG2a ratio); a positive slope indicates a Th2 biased immune response. (B) Shows for comparison purposes, the IgGl/IgG2a ratios obtained with OVA alone and
with either Quil A, 10 µg, or alum, 300 µg. The decrease in the IgGl/IgG2a ratio from sera of mice immunized with Quil A indicates a Th1 immune response, while the absence of change in the same ratio from sera of mice immunized with alum indicates a Th2 immune response.

Fig. 6 shows the effects of increasing doses of DS-QS, 10, 50 and 250 µg, on the OVA-stimulated proliferative response of spleenocytes isolated from mice immunized with OVA and the different doses. For comparison purposes, the proliferative response of spleenocytes isolated from mice immunized with OVA alone (— ), with Quil A, 10 µg (— ) or alum, 300 µg (······) are indicated.

Fig. 7 shows the effects of different adjuvants on the secretion of IFN-γ by spleenocytes isolated from mice immunized with OVA plus alum DS-QS (10, 50 or 100 µg). For comparison purposes the IFN-γ secreted by OVA alone, with alum (300 µg) or Quil A (10 µg) are shown. The secreted IFN-γ stimulated by DS-QS was similar to that stimulated by alum. The produced IFN-γ was measured by ELISA after growing the cells in the presence of OVA (10 µg/mL), for 24 and 48 hours.

Fig. 8 shows the effects of alum, Quil A (QS), and DS-QS on the production of antigen-specific CTL. C57BL/6 mice were immunized s.c. on days 1, 15 and 29 with OVA (10 µg) alone or in combination with one of the following compounds: alum (300 µg), QS (10 µg) and DS-QS (10 or 50 µg). On day 43, spleens were removed and the CTL activity at various effector:target (E:T) cell ratios determined as described in Example 10.

DETAILED DESCRIPTION OF THE INVENTION

As far as it is known, the only proposed use for DS-QS has been that of an excipient for saponin adjuvants, such as QS-7, QS-18 and QS-21, but not as an adjuvant (US 6,645,495 Bl). In publication US 2005/0191310 A1, the use of QS-7 as an adjuvant is proposed once more, but only when administered with certain excipients described in the application, DS-QS being one of the proposed excipients. Consequently, that DS-QS can elicit an effective Th2 immunity and not act just as an excipient, has important implications for AD vaccines, where a Th2 immunity is required to avoid damage to the CNS, but, there is also a critical need to deliver a co-stimulatory signal to compensate for the immune senescence’s detrimental effects on the APCs and T cells. Because of immune senescence, the need to counterbalance the
effects associated with aging is not limited to sub-unit antigen vaccines, but it also
applies to DNA and VLP vaccines.

[0052] In effect, there are two unrelated events associated with the later vaccines, the
first would be the expression by engineered cells of the antigen, either as a protein or
incorporated in a VLP, and the second would be the response that the immune system
would mount against the newly expressed antigen(s). While it is unlikely that antigen
expression induced by DNA and VLP vaccines would be affected by aging, the
immune response would be impacted by the immune system performance, which
deteriorates as a result of the aging process. Thus, the use of natural or synthetic non-
acylated bidesmosidic triterpene saponins carrying an aldehyde or ketone group
would be advantageous for AD vaccines, such as Aβ or tau-based, because of the i)
stimulation of a polarized Th2 immunity that is independent of the presence of T cell
epitopes in the antigen, ii) amelioration of the immune senescence’s effects by
delivering an alternative co-stimulatory signal that prevents T cell anergy, hi) their
very low toxicity and iv) a good stability that would assure a long shelf life.

[0053] The methods of the present invention comprise of administering to an
individual for preventive or therapeutic purposes a vaccine formulation composed of
an Aβ or tau-derived antigen, or a combination of both types of antigens, which can
be synthetic, produced by recombinant DNA technology or natural product, and that
is selected from the group consisting of i) Aβ-protein (1-40), (1-42) and (1-43); ii)
Aβ-protein (1-40), (1-42) and (1-43), but without the dominant T cell epitope
sequences 16-33 and/or 28-42 (Monsonego A et al. J Clin Invest. 112:415-422, 2003);
iii) Aβ-peptide (1-15) carrying the Aβ-protein B cell epitopes; iv) Aβ-peptide (1-6)
carrying a dominant B cell epitope, v) full length tau protein, vi) a peptide derived
from the amino acid sequence of the tau protein and that is or is not phosphorylated.
In one preferred embodiment the peptides are either free or linked covalently to a
carrier selected from the group consisting of a i) protein like KLH, tetanus toxoid,
edestin or albumin; ii) multiple antigenic peptide system or MAP (US 5,229,490),
such as a linear or branched oligolysine core; iii) peptide, like the linear PADRE T
helper epitope (US 8,232,373); v) liposome (US 2009/0202627 Al; 2011/0076323
A1) and v) viral like particle, such as a bacteriophage, tobacco mosaic virus (TMV) or
adenovirus. In another preferred embodiment, the Aβ or tau-derived antigens, free or
conjugated to a carrier, are formulated with a natural or synthetic saponins or saponin
analogs carrying an aldehyde or ketone group, that is non-acylated or de-acylated, and
that is selected from the group consisting of i) de-acylated quillaja saponins, DS-QS; ii) de-acylated saponins from *Silene jenissensis*; iii) squaroside A; iv) non-acylated saponins from *Gypsophila sp.* and v) other non-acylated bidesmosidic triterpene saponins that are equal or similar to DS-QS, such as those saponins from *Saponaria sp.* and other plant species. The formulation is substantially free from acylated saponin or acylated saponin analogs. As discussed above, when practicing this embodiment, it is important to employ a vaccine or formulation that includes an adjuvant that stimulates Th2 immunity but stimulates substantially no Th1 immune response. Therefore, the presence of more than minor amounts of acylated saponin or acylated saponin analog is to be avoided.

[0054] The present invention is also directed to pharmaceutical and veterinary compositions comprising of one or more non-acylated bidesmosidic triterpene glycosides or saponins carrying an aldehyde or ketone group, and one or more pharmaceutically acceptable diluents, carriers or excipients. These compositions may be employed as immune potentiators of Th2 immunity in animals and humans.

[0055] The present invention is also directed to preventive or therapeutic vaccines comprising one or more Aβ or tau-derived antigens, or combinations of both, and one or more non-acylated bidesmosidic triterpene glycosides or saponins having an aldehyde or ketone group.

[0056] The present invention is also directed to a method of eliciting and enhancing prophylactically or therapeutically a Th2 immune response in a mammal, comprising of administering an effective amount of one or more non-acylated bidesmosidic triterpene glycosides or saponins carrying an aldehyde or ketone group, to enhance the immune response of a mammal to one or more Aβ or tau-derived antigens, either separately or in combination.

[0057] The present invention is also directed to a method of preventive or therapeutic vaccination, comprising administering one or more Aβ or tau-derived antigens, or combinations of both types of antigens, and one or more non-acylated bidesmosidic triterpene glycosides or saponins having an aldehyde or ketone group.

[0058] The present invention is also directed to a method of preventive or therapeutic vaccination, comprising of one or more Aβ or tau-derived antigens, either separately or combinations of both, and non-acylated bidesmosidic triterpene glycosides or saponins having an aldehyde or ketone group, that can be administered parenterally, such as subcutaneous, intramuscular or intraperitoneal injection; mucosally, such as
nasally, sublingually or rectally; and transdermally, such as by electroporation and transdermal patch.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0059] A first aspect of the invention is directed to a vaccine formulation for human or veterinary use, comprising:

(a) one or more antigens selected from the groups consisting of a amyloid-β derived antigens and a tau-derived antigen, and

(b) one or more triterpene saponins adjuvants, wherein

(1) an aldehyde or ketone group is present on the triterpene aglycone core structure of the triterpene saponin,

(2) linear or branched oligosaccharides are attached to positions 3 and 28 of the triterpene aglycone core structure, and

(3) the glycosyl residues of the oligosaccharide at position 28 are non-acylated.

[0060] In a second aspect of the invention, the triterpene saponin adjuvant is selected from the group consisting of

(1) Quillaja desacysaponin, having a 3-glucuronic acid residue, a quillaic acid as triterpene aglycone core with an aldehyde group at position 4, and lacking an acyl group on the oligosaccharide at the position 28 of the triterpene aglycone,

(2) Gypsophila sp. saponins, having a 3-glucuronic acid residue, a gypsogenin as triterpene aglycone core with an aldehyde group at position 4, and lacking an acyl group on the oligosaccharide at position 28 of the triterpene aglycone,

(3) Acanthophyllum sp. saponins, having a 3-glucuronic acid residue, a gypsogenin as triterpene aglycone core with an aldehyde group at position 4, and lacking an group on the oligosaccharide at position 28 of the triterpene aglycone,

(4) Saponaria sp. saponins having a 3-glucuronic acid residue, a gypsogenin as triterpene aglycone core with an aldehyde group at position 4, and lacking an acyl group on the oligosaccharide at position 28 of the triterpene aglycone, and

(6) Saponins from Silenejenisseensis having a 3-glucuronic acid residue, a quillaic acid as triterpene aglycone core with an aldehyde group at position 4, and lacking an acyl group on the oligosaccharide at the position 28 of the triterpene aglycone.
A third aspect of the invention is directed to the vaccine formulation of aspect 2, wherein the 3-glucuronic acid residue is either alone or linked to a linear or branched oligosaccharide having from one to four glycosyl residues.

A fourth aspect of the invention is directed to a vaccine formulation of aspect 2 or 3, wherein the oligosaccharide at position 28 is a linear or branched oligosaccharide having from two to seven glycosyl residues.

A fifth aspect of the invention is directed to a vaccine formulation of aspects 1, 2, 3 or 4 wherein one or more of the triterpene saponins is natural, semi-synthetic or synthetic.

A sixth aspect of the invention is directed to a vaccine formulation of any of aspects 1 through 5, wherein said amyloid-β derived antigen is a peptide having the amino acid sequence corresponding to amino acid residues 1 to 40 or 1 to 42.

A seventh aspect of the invention is directed to a vaccine formulation of claim of any of aspects 1 through 5, wherein said amyloid-β derived antigen is a peptide having one or more amino acid sequences found in the amino acid sequence of 1 to 42.

An eighth aspect of the invention is directed to a vaccine formulation of aspect 7, wherein said amyloid-β derived antigen is a peptide covalently linked to a carrier, such as a peptide, a protein, a viral like particle, a liposome, an immune stimulating complex (ISCOM), or a nanoparticle formed by cross-linked sugars.

A ninth aspect of the invention is directed to a vaccine formulation of any of aspects 1 through 5, wherein said tau protein derived antigen is a peptide having the amino acid sequence corresponding to residues 1 to 352 or 1 to 441 from one of six tau isoforms.

A tenth aspect of the invention is directed to a vaccine formulation of aspect 9, wherein said tau-derived antigen is a peptide that is phosphorylated at serine, threonine or tyrosine amino acid residues.

An eleventh aspect of the invention is directed to a vaccine formulation of aspect 10, wherein said tau-derived antigen is a peptide covalently linked to a carrier, such as a peptide, protein, viral like particle, liposome, ISCOM or a nanoparticle formed by cross-linked sugars.

A twelfth aspect of the invention is directed to a vaccine formulation of aspect 5, further comprising a pharmaceutically acceptable carrier, diluent or another Th2 adjuvant.
A thirteenth aspect of the invention is directed to a method of vaccinating a subject, comprising administering the vaccine of aspect 7 to said subject to potentiate a Th2 immune response in said subject to said antigen.

A fourteenth aspect of the invention is directed to a method of vaccinating a subject, comprising administering the vaccine of aspect 8 to said subject to potentiate a Th2 immune response in said subject to said antigen.

A fifteenth aspect of the invention is directed to a method of vaccinating a subject, comprising administering the vaccine of aspect 10 to said subject to potentiate a Th2 immune response in said subject to said antigen.

A sixteenth aspect of the invention is directed to a method of vaccinating a subject, comprising administering the vaccine of aspect 11 to said subject to potentiate a Th2 immune response in said subject to said antigen.

As discussed above, vaccine formulations are substantially free of acylated saponins or acylated saponin analogs. Preferably, the vaccine formulations have less than 3%, less than 1% or less than 0.5% by weight of acylated saponins or acylated saponin analogs based upon total weight of saponin or saponin analog.

Vaccine: As used herein, the term "vaccine" refers to a formulation which contains the composition of the present invention and which is in a form that is capable of being administered to an animal, preferably a human. Typically, the vaccine comprises a conventional saline or buffered aqueous solution medium in which the composition of the present invention is suspended or dissolved. In this form, the composition of the present invention can be used conveniently to prevent, ameliorate, or otherwise treat a condition. Upon introduction into a host, the vaccine is able to provoke an immune response.

Vaccine compositions of the invention comprise, or alternatively consist of, an immunologically effective amount of the inventive immune enhancing composition together with a pharmaceutically acceptable diluent, carrier or excipient.

All patents and publications referred to herein are expressly incorporated by reference in their entirety.

EXAMPLES

The following examples are illustrative, but not limiting, of the compositions and methods of the present invention. Other suitable modifications and adaptations of
the variety of conditions and parameters normally encountered and obvious to those skilled in the art are within the spirit and scope of the invention.

**Example 1**

*De-acylation of quillaja saponins (q. saponins)*

A q. saponins fraction containing the components from QS-16 to QS-21 is obtained by purification of an enriched q. saponins preparation prepared by extensive dialysis of an aqueous bark extract against water on a semi-preparative C-18 column. The different fractions obtained by stepwise elution with acetonitrile/water containing 0.1% TFA, are analyzed by HPLC using a Vydac C-4 column and an acetonitrile/water linear gradient containing 0.1% TFA (Kensil CR et al, *J Immunol.* 146:431-437, 1991) and those containing QS-16 to QS-21 are pooled. The solvent of these pooled fractions is evaporated and the syrupy residue dissolved in water and lyophilized. Alternatively, the whole QS preparation may be used. Removal of the acyl group from the fucose of the C-28 oligosaccharide of the pooled q. saponins fraction can be accomplished by treatment with a solution of 0.2N sodium hydroxide in 90% 1-propanol (US 5,443,829), or as described here with a solution of ammonium hydroxide in methanol (MeOH) (DJ Marciani et al. *Vaccine* 2000; 18:314-3151). To 1 g of QS in a 100 ml. round bottom flask, add with stirring 30 mL of a 3 N ammonium hydroxide in 80% MeOH, the flask is close tightly and the stirring is continued for about 24 hours at RT. Filter out the insoluble materials from the cloudy solution using a fine glass filter paper, wash the insoluble residue with 7 mL of MeOH and pool the filtrate and wash. Monitor the de-acylation by HPLC using a Vydac C-4 column edited with a waier-acetomitrile (ACN) gradient with 0.1% TFA. De-acylation is complete when the original starting material’s HPLC profile is replaced by a small number of peaks, about 4, that elute close to the front. The DS-QS have two oligosaccharide side-chains, a C-3 disaccharide or trisaccharide and a C-28 tetrasaccharide, pentasaccharide or hexasaccharide, the major components with a mol. wt. (MW) of ~ 1,536 and 1,700. Evaporate the MeOH-ammonium hydroxyde from the clear filtrate in a rotary evaporator or a centrifugal vacuum concentrator; dissolve the syrupy residue in 20 mL of de-ionized water, and evaporate again to remove residual MeOH. Dissolve the syrupy residue with 10-15 ml of de-ionized water and lyophilize it, to yield the DS-QS as an off-white powder. The yield is ~ 40 to 70% of
the initial QS material and includes both the DS-QS and the fatty acids produced
during deacylation. Dissolve the dry product in 12 mL of water, and adjust the pH to
3.8 with acetic acid; to this solution, add with mixing 12 mL of 1-propanol to yield a
50% concentration of the alcohol. Adsorb this solution to 2 - 2.5 g of silica gel
(Lichropep Si60 or similar one) and evaporate the solvent under a gentle stream of dry
nitrogen or under vacuum. Load the pre-adsorbed silica evenly on top of a silica gel
column made of Lichropep Si60 or a similar one (2.5 cm ID x 14 cm L; ~ 70 mL) and
elute by gravity with ~ 70 mL of 1-propanol, 3 column volumes of 85% 1-propanol,
2.5 column volumes of 80% 1-propanol and 75% 1-propanol respectively and 1
column volumes of 50% 1-propanol. Collect 30 mL fractions and analyze them by
RP-HPLC to identify the different DS-QS components. Those corresponding to QS-
18 and QS-21 and eluting together with the 75% 1-propanol, are evaporated in a
centrifugal vacuum concentrator to remove the solvent, dissolved in water and
lyophilized. The dry DS-QS, free of fatty acids and some other minor components, is
stored at RT under vacuum and over a desiccant to remove any water from the
preparation. The DS-QS purified fraction is analyzed by reverse phase HPLC on C-18
and its molecular weight profile determined by MALDI.

Example 2

Purification of saponins from Gypsophila sp

Five g of a crude preparation of gypsophila saponins are extracted with 100
mL of 75% ethanol (EtOH) for 4 hours under reflux, and the solvent is evaporated
under vacuum in a rotary evaporator. Suspend the dry residue in ~ 75 mL of water
and extract sequentially with 75 mL of EtOAc and 75 mL of n-butanol (n-BuOH);
after shaking the n-BuOH and aqueous solution, the two solvents are allowed to
separate overnight at RT. The n-BuOH phase that contains the bidesmosidic saponins,
some of which may carry a p-methoxycinnamoyl residue (Yao S et al. Chin J Nat
Med. 8:28-33, 2010), is evaporated under reduced pressure to recover the mixed
saponins. The n-BuOH soluble saponins are fractionated by silica gel chromatography
on Si 60, 230-400 mesh, equilibrated with chloroform/methanol/water,
(CHCVMethOH/I-LO), 100:10:1, the saponins sample is diluted in 35 ml of the same
solvent, applied to the silica gel column, which is eluted stepwise, with 90:10:1,
pure MeOH. Analyze the fractions collected by HPLC using a C-18 column and a linear gradient of MeOH/water, 20% to 80% MeOH and monitored by measuring the absorbance at 210 nm. Pool those fractions containing specific saponins and determine their UV/VIS absorption spectra between 230 nm and 340 nm, samples showing absorbance peaks in this region are rejected due to the presence of p-methoxycinnamoyl residues in the saponins. Evaporate the solvent in a rotary evaporator and purify the saponins to homogeneity by using a preparative C-18 column eluted with a linear gradient of MeOH/water, from 20% to 80% MeOH. Pool the samples containing the saponin, evaporate the MeOH solvent under reduced pressure, dissolve the residue in water and lyophilize to obtain the purified Gypsophila saponin (Fig. 3). Determine the M.W. of each non-acylated bidemosidic saponin component by MALDI.

Example 3

*Testing of immune stimulatory effects using anti-OVA IgG titers from immunized mice sera*

[0082] Adjuvant effects can be determined by measuring anti-OVA antibody titers by ELISA following immunization with OVA in the absence and presence of adjuvant candidates as follows: BALB/c mice (8-10 weeks old) are immunized subcutaneously three times at two week intervals with one of the following formulations: 10 µg of OVA (Sigma) alone or plus i) an adjuvant of the present invention, 10 to 250 µg doses, or ii) Quil A, 10 µg dose. Control mice are injected with PBS, PBS with OVA or PBS with OVA plus 300 µg of aluminum hydroxide. Sera are harvested two weeks after the last immunization and the anti-OVA antibody titers determined by ELISA using Immunon II plates coated overnight at 4°C with 100 µL of an OVA solution (50 µg/ml in PBS). Plates are washed twice with PBS and nonspecific binding prevented by incubating for 1.5 h at 37°C with 100 µL of 2% casein acid hydrolysate in PBS per well, followed by four washes with 0.05% Tween 20 in distilled water. Sera dilutions from 1:20 to 1:1,562,500 in PBS (100 µL, per well) are incubated for 1 h at RT. Plates are washed with 0.05% Tween 20 in distilled water and delivered 100 µL per well of a horse radish peroxidase (HRP) conjugate of goat anti-mouse IgG or IgGl, IgG2a or IgG2b in 2% casein acid hydrolysate diluent and incubated for 30 min at room temperature. Plates are then washed as described before and the extent of peroxidase
reaction determined by incubating with 3,3',5,5'-tetramethylbenzidine (TMB) for 10 min at RT, stopping the reaction with 1 N sulfuric acid and measuring the absorbance at 450 nm subtracting the absorbance of the antigen-negative well from the absorbance of each antigen-positive well. The anti-OVA IgG1, IgG2a and IgG2b, or immune responses are determined from the end-point titers obtained by plotting the absorbance due to antigen-specific antibody binding as a function of the logarithm of the serum dilution, and the end-point titer estimated from the serum protocol to measure the immune stimulatory effect of Quil A, DS-QS and alum on the production dilution yielding an absorbance of 0.25 (cutoff value). Fig 4 illustrates the results of use of this of the different IgG isotypes by BALB/c mice.

Immune stimulatory agents or adjuvants affect the expression of the different antigen-specific IgG isotypes and in some cases the increase of a specific isotype can be used as an indicator or the immunity type: Th1 or Th2. In BALB/c mice production of IgG2a is dependent on the expression of IFN-γ, a Th1 immunity cytokine, thus increase or decrease of this isotype titers in this mouse strain can be a reliable indicator of the type of immunity stimulated by an adjuvant. The relative increase or decrease of IgG2a production can be assessed by plotting either the IgG1/IgG2a or IgG2a/IgG2a end-point titers ratio as a function of the adjuvant dose. An increase in IgG2a levels, as shown by a negative slope with increasing adjuvant doses, indicates Th1 immunity, while a positive slope would signify an increase of IgG1 production, an isotype associated to Th2 polarized immunity. Figure 5-A shows the use of this method to establish that DS-QS stimulate Th2 immunity; however, Quil A (Fig 5-B), as shown by the decrease in the log10 of the IgG1/IgG2a ratios determined at a 0 and 10 µg doses, stimulates Th1 immunity against OVA, an antigen that has both T and B cell epitopes.

Example 4

Effects of DS-QS and QuilA on lymphoproliferation stimulated by OVA

Single cell suspensions of spleens from control and immunized mice are prepared in complete medium, which consists of RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 10 raM HEPES buffer, 50 mM 2-mercaptoethanol and antibiotics, for cellular assays. Spleen cells, 2 x 10^5 per well, from control and immunized mice are cultured in triplicate in 0.2 mL of complete medium in 96-well
flat bottom microtiter plates with either medium, 10 µg/mL of OVA or 3 µg/mL of concanavalin A. Cultures are incubated at 37°C in a humidified, 5% CO2 incubator for 3 days, pulsed with [³H]thymidine, [³H]TdR, for 16 hours, and harvested using a Skatron (Sterling, VA) semi-automated harvester. Proliferation is determined from the [³H]TdR incorporated and measured by determining the radioactivity in a liquid scintillation counter. Fig 6 shows the negligible [³H]TdR incorporation in cells derived from mice immunized with OVA and DS-QS, in contrast to those cells derived from animals immunized with OVA and Quil A.

**Example 5**

**Determination of DS-QS and Quil A effects on IFN-γ αη à IL-4 cytokine production**

[0085] IFN-γ and IL-4 are cytokines associated with Th1 and Th2 immunity respectively that are produced by antigen-activated T lymphocytes in cell culture and can be determined by ELISA. Mice immunized 3 times every 2 weeks are sacrificed 2 weeks after the last immunization, their spleens harvested and single cell suspensions prepared in complete medium (Marciani DJ et al. Vaccine 18:3141-3151, 2000). As negative controls spleen cells from mice injected with PBS only are used. Cells (2 x 10^5 per well) are cultured in 96-wells flat microtiter plates (Costar) with either medium alone or medium containing OVA concentrations ranging from 0.5 to 5 pg/mL (to activate lymphocytes) at 37°C in humidified air with 5% CO₂ for 18, 24 or 48 hours. After the specified incubation time the media is removed, centrifuged to take out cellular debris and the clear supernatant frozen and stored at - 20°C for subsequent assays. Levels of IFN-γ and IL-4 are determined using commercial ELISA kits (Endogen; BD-Pharmigen) according to their instructions and the cytokine levels expressed in pg/mL. Fig. 7 shows the effects of DS-QS and Quil A on the secretion of IFN-γ, confirming that DS-QS stimulates a Th2 immune response.
Example 6

**Formulations for increasing the production of antibodies specific for Λβ antigens**

A peptide with the amino acid sequence of Λβ40, Λβ42 or Λβ43, prepared by organic synthesis, is dissolved in physiological phosphate buffered saline solution (PBS) containing 2% mannitol as an stabilizing agent, to give concentrations of 50, 100, 200 and 400 µg/mL of the Λβ antigen. To each of these solutions add an equal volume of PBS containing 40, 200, 400 and 1000 µg/mL of DS-QS, mix and sterilize by filtration; the final solutions having 25, 50, 100 and 200 µg/mL of the Λβ antigen, would also contain each 20, 100, 200 or 500 µg/mL of DS-QS. As controls for Th1 and Th2 immune responses, the Λβ antigen(s) would be formulated with 50 µg/mL of Quil A and 400 µg/mL of alum, respectively. The sterile samples containing DS-QS are stored at 4°C. For evaluation of the different vaccine formulations, 0.5 mL doses are administered intraperitoneal (i.p) to female BALB/c mice, 7-8 weeks old, on days 1, 15 and 30. Blood samples are collected on days 14, 29 and 44, sera is prepared and store frozen for subsequent ELISA determinations; the animals are sacrificed on day 44. The total antibodies and their isotypes IgGl, IgG2a and IgG2b present in sera were determined by ELISA using Immulon II plates coated overnight at 4°C with 100 µL of an Λβ-antigen solution containing 50 µg/ml in PBS. Plates with aggregated Λβ were prepared by delivering to ea. well 50 µL of the Λβ antigen, 100 µg/ml in water, followed by 50 µL of 0.3 M NaCl in 20 mM Na phosphate buffer pH 7.2, mixing the solutions and coat overnight at 4°C. After the coating procedure, treat the plates as indicated above. Plates are washed twice with PBS and nonspecific binding prevented by incubating for 1.5 h at 37°C with 100 µL of 2% casein acid hydrolysate in PBS per well, followed by four washes with 0.05% Tween 20 in distilled water. Sera dilutions from 1:20 to 1:1,562,500 in PBS (100 µL per well) are incubated, 1 h at RT, washed, added HRP conjugates of anti-mouse goat IgG and processed and the data analyzed as described in Example 3.
Example 7

**Formulations for increasing the production of antibodies specific for hyperphosphorylated tau-derived peptide antigens**

[0087] A peptide with the amino acid sequence of Tau379-408[P-Ser\textsubscript{944,4}] or Tau417-427[P-Ser\textsubscript{42,4}], prepared by organic synthesis, free or conjugated to KLH via a linker at the amino terminal, is dissolved in physiological phosphate buffered saline solution (PBS) containing 2% mannitol as an stabilizing agent, to give concentrations of 50, 100, 200 and 400 µg/mL of the tau-derived antigen. To each of these solutions add an equal volume of PBS containing 40, 200, 400 and 1000 µg/mL of DS-QS, mix and sterilize by filtration; the final solutions having 25, 50, 100 and 200 µg/mL of the tau antigen, would also contain each 20, 100, 200 or 500 µg/mL of DS-QS. As controls for Th1 and Th2 immune responses, the tau antigen(s) would be formulated with 50 µg/mL of Quil A and 400 µg/mL of alum, respectively. The sterile samples containing DS-QS are stored at 4°C. For evaluation of the different vaccine formulations, 0.5 mL doses are administered intraperitoneal (i.p) to female BALB/c mice, 7-8 weeks old, on days 1, 15 and 30. Blood samples are collected on days 14, 29 and 44, sera is prepared and store frozen for subsequent ELISA determinations; the animals are sacrificed on day 44. The total antibodies and their isotypes IgGl, IgG2a and IgG2b present in sera were determined by ELISA using Immulon II plates coated overnight at 4°C with 100 µL of a solution of a conjugated tau-antigen to BSA, containing 50 µg/ml of the tau antigen in PBS. Plates are washed twice with PBS and nonspecific binding prevented by incubating for 1.5 h at 37°C with 100 µL of 2% casein acid hydrolysate in PBS per well, followed by four washes with 0.05% Tween 20 in distilled water. Sera dilutions from 1:20 to 1:1,562,500 in PBS (100 µL per well) are incubated, 1 h at RT, washed, added HRP conjugates of anti-mouse goat IgG and processed and the data analyzed as described in Example 3.

Example 8

**Effects of non-ionic polysorbate detergents on the formulations that increase the α\textgammaθ-β or anti-tau immune response**

[0088] To determine the role of non-ionic polysorbate detergents on the immune stimulatory effects of DS-QS and Quil A, the formulations from Examples 6 and 7 are
used for immunization, but in the presence of 4 or 8 mg/mL of Tween-80 or polysorbate 80, (polyoxyethylene (20) sorbitan monoooleate). Following the immunization schedules from Examples 6 and 7, total IgG and the isotypes IgG1, IgG2a and IgG2b titers, are determined by ELISA and analyzed following the protocol described in Example 3.

Example 9

Effects of DS-QS and QuilA on the lymphoproliferation stimulated by Aβ or tau-derived antigens

Animals from Examples 6 and 7, are sacrificed on day 44 and used to prepare single cell suspensions of spleens from control and immunized mice in complete medium, which consists of RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 10 mM HEPES buffer, 50 mM 2-mercaptoethanol and antibiotics, for cellular assays. Spleen cells, 2 x 10^5 per well, are cultured in triplicate in 0.2 mL of complete medium in 96-well flat bottom microtiter plates with either plain medium, or containing 20 μg/mL of Aβ or tau-derived antigens or 4 μg/mL of con A. Cultures are incubated at 37°C in a humidified, 5% CO2 incubator for 3 days, pulsed with [³H]thymidine, [³H]TdR, for 16 hours, and harvested using a Skatron (Sterling, VA) semi-automated harvester. Proliferation is determined from the [³H]TdR incorporated and measured by determining the radioactivity in a liquid scintillation counter.

Example 10

Effects of DS-QS on the production of OVA specific CTLs

A reliable indicator of Th1 immunity is the antigen-specific CTL production against cells expressing the antigen used as an immunogen processed by the class I pathway for presentation. Availability of the EL4 mouse cell line (C57BL/6 mice) transfected with the OVA gene, E.G7-OVA (MW Moore et al. Cell 54:777-785, 1988), allows such assessment. Spleen cells (2 x 10^6 cells/well) from C57BL/6 mice immunized i.p. according to the protocol from Example 3 as well as from non-immunized mice and sacrifice on day 44, are added to 24 well plates containing 1 mL of complete medium plus 1 x 10^5 E.G7-OVA cells irradiated with X rays (20,000 R) to stop cell division and incubated for 6 days to yield the effector (E) cells. Target
cells (T) are the EL4 (negative control) and E.G7-OVA cells expressing the OVA gene that have been previously incubated for 1 hour with 300 μCi of $^{51}$Cr labeled sodium chromate and washed. Target cells ($1 \times 10^6$) are incubated with an increasing number of E cells for 6 h, and the amount of $^{51}$Cr released in the supernatant (result of cell lysis by CTLs) measured in a liquid scintillation counter and expressed in counts per min (cpm). As a control for total lysis, i.e. 100% or maximum release, use $^{51}$Cr labeled E.G7-OVA target cells treated with 2% Triton x-100; the negative controls are $^{51}$Cr labeled E.G7-OVA cells treated with medium only and EL4 cells (spontaneous release). Calculate the percent lysis for each E:T ratio using the following formula:

$$\text{% lysis} = \frac{(\text{cpm lysis by CTLs} - \text{cpm spontaneous release})}{(\text{cpm 100% lysis} - \text{cpm spontaneous release})}$$

Plot per cent lysis as a function of the E:T ratio, the positive slope would be proportional to the CTLs' lytic activity. The lysis of EL4 cells by OVA-specific CTLs should be equal to the spontaneous release.

**Example 1**

**Formulations for increasing the production of antibodies specific for Aβ-derived antigens**

An Aβ-derived peptide with an amino acid sequence such as Aβ1-7 (US 8,034,348 B2); Aβ1-6 attached to a VLP (US 7,279,165 B2); the N-end terminal Aβ42 sequence DAEFH or its mimotopes, free or conjugated to an acceptable carrier (US 8,022,180 B2); Aβ10-21 in αl-D or αl-L configurations, with a N-terminal and/or C-terminal substituent (US 2002/0094335 A1), prepared by organic synthesis, is dissolved in PBS containing 2% mannitol as an stabilizing agent, to give concentrations of 50, 100, 200 and 400 μg/mL of the Aβ antigen. These vaccine formulations are administered i.p. to BALB/c mice, without or with 4 or 8 mg/mL of polysorbate 40, plus 20, 100, 200 or 500 μg/mL of DS-QS. As a Th1 control, the animals are vaccinated with a similar formulation, but with 50 μg/mL of Quil A instead of DS-QS. Process the samples by ELISA as described in Example 6 for Aβ-antigens, and analyze the data as described in Example 3 by plotting IgG1/IgG2a or IgG1,α/β/IgG2a titers ratio as a function of the adjuvant dose.
Example 12

Effects of D S-QS and Quil A on the immune response against Aβ-derived antigens of human APP transgenic mice

Eight months old female APP transgenic mice carrying a human amyloid precursor protein (APP) are injected i.p. with the vaccine formulation as described in Example 6, bleed from the tail vein at days 14, 29 and 44, and after at months 2, 3, 5, 6 and 8 after the first immunization and the antibody titers for each isotype determined by ELISA as described in Example 6. The affinities of the different antibody isotypes for the Aβ-derived antigens are determined at the same time intervals indicated above using the avidity index, an indicator of the average antibody affinity, measured by ELISA utilizing the ammonium thiocyanate elution method (Pullen GR et al. J Immunol Methods 86:83-87, 1986; Marciani DJ et al. Vaccine 9:89-96, 1991). Briefly, a high binding surface microtiter plate, e.g. Immulon 4HBX or Enhanced Binding plates, both from Thermo Electron Corp. are coated with Aβ-derived antigens as described in Example 6, and the wells are blocked with 10% normal goat serum and washed with 0.05% Tween-20 in water. Immune mouse sera diluted in 10% normal goat serum in PBS to a titer resulting in an A450 of 0.5 - 1.0 in a standard ELISA were incubated in ea. well for 1 hour at RT. Well are washed with 0.05% Tween-20 in water and then incubated with concentrations of ammonium thiocyanate in the range of 0 to 4 M in 0.1 M Na phosphate, pH 6. Remove the thiocyanate solution, wash with 0.1 M Na phosphate buffer, and measure the mouse IgG bound to the Aβ-derived antigen using a goat anti-mouse IgG-HRP conjugate and TMB as a chromogenic substrate. The avidity index, determined from a plot of thiocyanate concentration versus A450, is expressed as the molarity of a thiocyanate solution that removes enough anti-Aβ antibodies to reduce by 50% the absorbance measured in the absence of incubation with ammonium thiocyanate.
WHAT IS CLAIMED IS:

1. A vaccine formulation for human or veterinary use, comprising
   (a) one or more antigens selected from the groups consisting of a amyloid-β derived antigens and a tau-derived antigen, and
   (b) one or more triterpene saponins adjuvants, wherein
      (1) an aldehyde or ketone group is present on the triterpene aglycone core structure of the triterpene saponin,
      (2) linear or branched oligosaccharides are attached to positions 3 and 28 of the triterpene aglycone core structure, and
      (3) the glycosyl residues of the oligosaccharide at position 28 are non-acylated.

2. The vaccine formulation of claim 1, wherein said triterpene saponin adjuvant is selected from the group consisting of
   (1) Quillaja desacysaponin, having a 3-glucuronic acid residue, a quillaic acid as triterpene aglycone core with an aldehyde group at position 4, and lacking an acyl group on the oligosaccharide at the position 28 of the triterpene aglycone,
   (2) Gypsophila sp. saponins, having a 3-glucuronic acid residue, a gypsogenin as triterpene aglycone core with an aldehyde group at position 4, and lacking an acyl group on the oligosaccharide at position 28 of the triterpene aglycone,
   (3) Acanthophyllum sp. saponins, having a 3-glucuronic acid residue, a gypsogenin as triterpene aglycone core with an aldehyde group at position 4, and lacking an group on the oligosaccharide at position 28 of the triterpene aglycone,
   (4) Saponaria sp. saponins having a 3-glucuronic acid residue, a gypsogenin as triterpene aglycone core with an aldehyde group at position 4, and lacking an acyl group on the oligosaccharide at position 28 of the triterpene aglycone, and
   (5) Saponins from Silenejenisseensis having a 3-glucuronic acid residue, a quillaic acid as triterpene aglycone core with an aldehyde group at position 4, and lacking an acyl group on the oligosaccharide at the position 28 of the triterpene aglycone,
3. The vaccine formulation of claim 2, wherein the 3-glucuronic acid residue is either alone or linked to a linear or branched oligosaccharide having from one to four glycosyl residues.

4. The vaccine formulation of claim 2, wherein the oligosaccharide at position 28 is a linear or branched oligosaccharide having from two to seven glycosyl residues.

5. The vaccine formulation of claim 1, wherein one or more of the triterpene saponins is natural, semi-synthetic or synthetic.

6. The vaccine formulation of claim 1, wherein said amyloid-β derived antigen is a peptide having the amino acid sequence corresponding to amino acid residues 1 to 40 or 1 to 42.

7. The vaccine formulation of claim 1, wherein said amyloid-β derived antigen is a peptide having one or more amino acid sequences found in the amino acid sequence of 1 to 42.

8. The vaccine formulation of claim 7, wherein said amyloid-β derived antigen is a peptide covalently linked to a carrier, such as a peptide, a protein, a viral like particle, a liposome, an immune stimulating complex (ISCOM), or a nanoparticle formed by cross-linked sugars.

9. The vaccine formulation of claim 1, wherein said tau protein derived antigen is a peptide having the amino acid sequence corresponding to residues 1 to 352 or 1 to 441 from one of six tau isoforms.

10. The vaccine formulation of claim 9, wherein said tau-derived antigen is a peptide that is phosphorylated at serine, threonine or tyrosine amino acid residues.

11. The vaccine formulation of claim 10, wherein said tau-derived antigen is a peptide covalently linked to a carrier, such as a peptide, protein, viral like particle, liposome, ISCOM or a nanoparticle formed by cross-linked sugars.
12. The vaccine formulation of claim 5, further comprising a pharmaceutically acceptable carrier, diluent or another Th2 adjuvant.

13. A method of vaccinating a subject, comprising administering the vaccine of claim 7 to said subject to potentiate a Th2 immune response in said subject to said antigen.

14. A method of vaccinating a subject, comprising administering the vaccine of claim 8 to said subject to potentiate a Th2 immune response in said subject to said antigen.

15. A method of vaccinating a subject, comprising administering the vaccine of claim 10 to said subject to potentiate a Th2 immune response in said subject to said antigen.

16. A method of vaccinating a subject, comprising administering the vaccine of claim 11 to said subject to potentiate a Th2 immune response in said subject to said antigen.
Fig. 1
Fig. 3
**Fig. 4**

A bar graph showing log₁₀ end-point anti-OVA IgG titers for different samples:
- OVA
- OVA-alum
- OVA-Quil A
- OVA DS-QSOVA
- DS-QSOVA 10 µg
- DS-QSOVA 50 µg
- DS-QSOVA 100 µg
- DS-QSOVA 250 µg

The graph compares IgG1, IgG2A, and IgG2B titers across these conditions.
Fig. 5A
Fig. 5B
Fig. 6
Fig. 7
Fig. 8
INTERNATIONAL SEARCH REPORT

INTERNATIONAL APPLICATION No.
PCT/US 14/48254

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/00; A61P 25/28; B01F 17/56 (2014.01)
CPC - A61K 39/000

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): A61K 39/000; A61P 25/28; B01F 17/56; C07K 7/06, 7/08, 14/435 (2014.01)
CPC: A61K 39/00; USPC: 424/185.1; 530/395

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched:

Electronic data base consulted during the international search

- MicroPatent; Google; Google Scholar; IP.com; PubMed; Qvantu Therapeutics, Marciani, vaccine, human, veterinary, animal, antigen, amyloid beta, tau, triterpene saponin, adjuvant, aldehyde, ketone, aglycone, oligosaccharide, are attached to positions 3 and 28 of the triterpene aglycone, non-acylated, glucuronic acid, Quillaja, desacysaponin, Gypsophila, Acanthophyllum, Saponaria, Silene

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>MARCIANI, DJ et al. Development of semisynthetic triterpenoid saponin derivatives with immune stimulating activity. Vaccine, Vol. 18, 2000, pp 3141-3151; page 3141, column 1, paragraph 1; page 3142, column 2, paragraphs 1-2; page 3145, figure 1</td>
<td>1-16</td>
</tr>
<tr>
<td>Y</td>
<td>US 2011/0177109 A1 (SMITH, III, GJ et al.) July 21, 2011; paragraphs [0009]-[0010], [0014], paragraph [0019], [0051], [0078], [0087], [0188], [0194], [0197], [0252], [0253], [0255], [0261], [0287]; paragraph [031] 1, page 4, line 30; claim 3</td>
<td>1-9, 1-15-16</td>
</tr>
<tr>
<td>A</td>
<td>US 6,262,029 B1 (PRESS, JB et al.) July 17, 2001; entire publication</td>
<td>1-16</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

- Special categories of cited documents:
  - 'A' - document defining the general state of the art which is not considered to be of particular relevance
  - 'E' - earlier application or patent but published on or after the international filing date
  - 'L' - document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  - 'O' - document referring to an oral disclosure, use, exhibition or other means
  - 'P' - document published prior to the international filing date but later than the priority date claimed

- Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

- Document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

- Document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Date of the actual completion of the international search: 22 September 2014 (22.09.2014)

Date of mailing of the international search report: 06 NOV 2014

Authorized officer: Shane Thomas

mail stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Form PCT/ISA/2 10 (second sheet) (July 2009)