Journal of Genomic Medicine and Pharmacogenomics
JGMP, 1(2): 225-232
www.scitcentral.com

ISSN: 2474-4670

Original Research: Open Access

Sphingosine-1-phosphate (S1P)-Containing Niosomes as Vehicle to Deliver Amyloid-β in a Novel Alzheimer´s Disease Vaccine

Iván Carrera[1*], Lucia Fernandez-Novoa[1], Carmen Vigo[2] and Ramón Cacabelos[3]

[1]Department of Health Biotechnology, EuroEspes Biotechnology 15165, Corunna, Spain

[2]*Atlas Pharmaceuticals, Sunnyvale94089, CA, USA*

[3]*EuroEspes Biomedical Research Center, Institute for CNS Disorders and Genomic Medicine, Corunna 15166, Spain*

ReceivedJuly 8, 2016; Accepted July 11, 2016; Published November 30, 2016

ABSTRACT

Vaccines against amyloid-beta-peptide (Aβ) have been widely investigated as a potential immunotherapeutic approach for Alzheimer´s disease (AD) during the past years. However, previous vaccines tested in mice and humans have failed to address the major hallmarks of AD, such as senile plaque-like Aβ deposits, plaque-associated dystrophic neurites and glial proinflammatory cytokines, due to massive T-cell activation that resulted in a meningoencephalitis-like reaction. We have recently developed a vaccine (EB101), based on passive immunotherapy, that delivered Aβ1-42 in a novel immunogen-adjuvant consisting of sphingosine-1-phosphate (S1P)-containing niosomes, to APP/PS1 transgenic mice before and after detectable AD-like pathological effects. Quantitative analysis of amyloid burden, performed in the affected brain regions, showed a notable decrease in the mean area of Aβ plaques when EB101 was compared with other treatments, demonstrating that S1P plays a key role as a regenerative-neuroprotective agent during the development and consolidation periods of AD neuropathology. In the present study, we screened the efficacy of each component of our immunogenic vaccine EB101 in the APP/PS1 transgenic mouse model, in order to characterize the positive effect of S1P in the immunotherapeutic response. Complementary results demonstrated that EB101 vaccine significantly prevents and reverses the AD neuropathology hallmarks by clearing Aβ plaques, reducing dystrophic plaque neurites and minimizing neuroinflammation, while markedly reducing neuronal degeneration and thus prolonging lifespan in the APP/PS1 transgenic mouse model, paving the way for future relevant clinical trials.

Keywords: Alzheimer's disease, Amyloid beta peptide, APP/PS1 transgenic mice, Immunotherapy, Vaccine

Abbreviations: AD: Alzheimer's Disease; **Aβ:** β-Amyloid Protein; **APP:** Amyloid Precursor Protein; **CA1:** Field CA1 Hippocampus; **CA3:** Field CA3 Hippocampus;**Cx:** Cortex; **DG:** Dentate Gyrus; **Ent:** Entorhinal Cortex; **GrDG:** Granular Dentate Gyrus; **IL:** Interleukin; **MODG:** Molecular Dentate Gyrus; **PoDG:** Polymorph Dentate Gyrus; **Tg:** Transgenic; **Th:** T Helper.

INTRODUCTION

In Western countries, Alzheimer's disease (AD) is the most frequent form of dementia, affecting up to one-third of elderly individuals [1]. Its pathological hallmarks in the brain are characterized by the accumulation of amyloid-β (Aβ) peptide amyloid plaques, neurofibrillary tangles with hyperphosphorylated tau proteins, neuronal-synaptic loss affecting particularly the neocortex, hippocampus and entorhinal regions [2]. Taking advantage of the potential aspects of the APP/PS1 double-transgenic mouse line, numerous investigations have used this particular model to study emergent therapies to prevent and reduce the neurodegenerative features of AD.

The purpose of the present study is to investigate the efficacy

Corresponding author: Iván Carrera, PhD.; Department Director of Health Biotechnology, EuroEspes Biotecnology 15165 – Corunna, Spain; Tel: +34 9817848484; Email:biotecnologiasalud@ebiotec.com

Citation: Carrera I, Fernández-Novvo oa L, Vigo C & Cacabelos R. (2016) Sphingosine-1-phosphate (S1P)-Containing Niosomes as Vehicle to Deliver Amyloid-β in a Novel Alzheimer´s Disease Vaccine. J Genomic Med Pharmacogenomics, 1(2): 225-232.

of each component of our immunogenic vaccine EB101 in the APP/PS1 transgenic mouse model, in order to characterize the positive effect of S1P in the immunotherapeutic response of AD-like pathology. This goal is intended to be achieved by neurohistochemical methods that will analyze the functionality of a new physiological adjuvant design, composed of naturally-occurring phospholipids that prove safe and efficacious in other types of vaccines (e.g. influenza), with an added biologically active phospholipid (S1P), known to stimulate an anti-inflammatory reaction and to act as a neuronal regenerating agent in vitro and in vivo studies [3]. Based on our previous studies, the preventive and therapeutic effect of the EB101 vaccine in APP/PS1 mice, designed to address the pitfalls of previous vaccines, conserve the immunogenicity targeted for the reduction of Aβ burden deposits, but avoid the massive activation of T-cell-mediated immune response that potentially causes adverse inflammatory effects. Moreover, this cost-effective vaccine was formulated to reduce Aβ burden by generating a robust anti-Aβ antibody production, in order to prevent or slow down the main AD-like pathological alterations and stimulating an anti-inflammatory T-helper (Th) 2 immune response.

MATERIALS AND METHODS

Experimental design

Two experimental treatment studies were carried out; one before AD onset, starting at 7 weeks of age (preventive treatment), and the other after AD onset at 35 weeks of age, when neuropathological AD characteristics were well established (therapeutic treatment). APPswe/PS1dE9 double-transgenic mice (B6C3F1/J), expressing a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and human presenilin 1 (PS1-dE9) mutants, were randomly divided into these two experimental studies, and in each study they were divided into four treatment groups (**Figure 1**), as follows: Preventive treatment: Group A was formed by 16 mice (12 transgenic and 4 wild-type mice; 12+4) that were immunized with a cocktail of synthetic human Aβ42/S1P-containing niosomes (EB101); group B, formed by 16 mice (12+4), immunized with Aβ42/liposome without S1P; group C, formed by 16 mice (12+4) immunized with S1P-containing niosomes; and group D, formed by 10 mice (6 transgenic and 4 wild-type mice, control) inoculated with PBS. Therapeutic treatment: The same treatments were administrated in groups A, B and C, formed by 16 mice in each group, while group D, formed by 10 mice inoculated with PBS, was the control. Mice were immunized with nine injections for seven months, inoculating 100µl containing a cocktail of Aβ (100µg) and phospholipid mix (1mg) per injection. A representation of the model is portrayed in **Figure 1.**

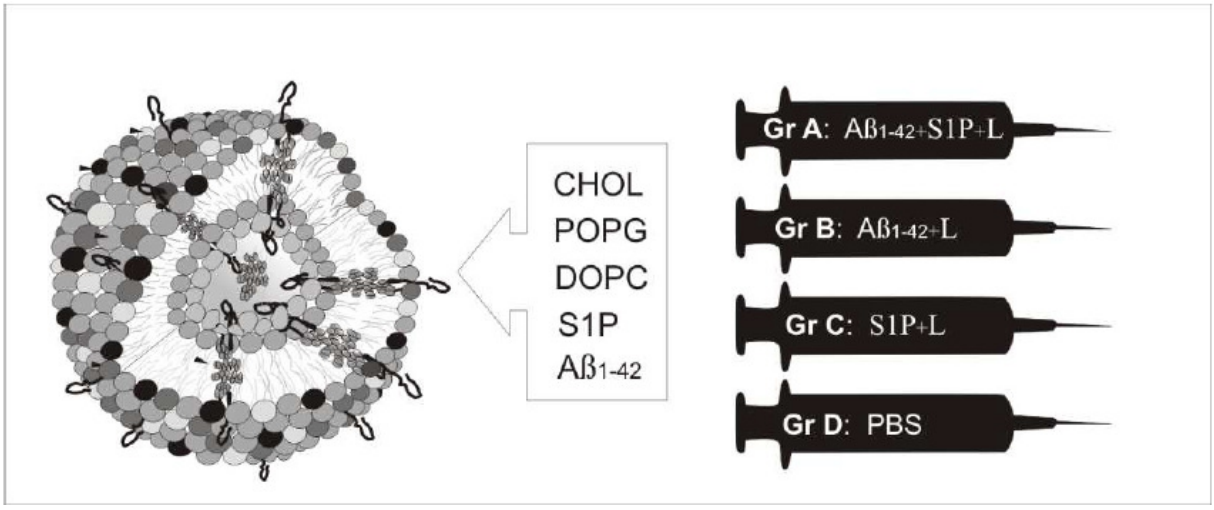

Figure 1. Structural characterization of the EB101 vaccine. Structural representation of Aβ1-42/S1P-containing liposome (EB101) and the treatment protocols of the four experimental mice groups.

Experimental procedure

Immunization procedures*:* 44 APPswe/PS1dE9 transgenic mice were inoculated intraperitoneally with 100 µl per injection of EB101 (group A), only liposome complex EB102 (group B) or PBS (group C), during seven months (9

injections) for each treatment period. The immunization protocol was systematically used in previous similar studies [4,5,6] and was followed strictly, with 3 injections every two weeks in the first month, and then one injection each following month. This immunization regimen was followed in the preventive and therapeutical periods.

Immunohistochemistry and imaging: Immunohistochemical analysis and imaging were prepared as previously reported [5,6].

Statistical analyses. All statistical parameters were performed with the use of SPSS (version 11.0; SPSS Inc, Chicago) and a P value <0.05 indicated statistical significance. The average range of Aβ plaque density and burden area were analyzed by using a two-factor repeated-measures analysis of variance (ANOVA) followed by a post hoc analysis when relevant. All data were expressed as the mean ± SEM.

RESULTS

Efficacy of S1P-containing niosomes (EB101) in preventing Aβ burden development

EB101 vaccine prevents and reverse AD neuropathology as we recently demonstrated, by the marked reduction of Aβ plaques, plaque-associated dystrophic neurites and astrocytosis in affected mouse brain areas. Next, we conducted an EB101 component screening study in order to elucidate the effective role of each in the prevention of Aβ deposits in the APP/PS1 mouse brain. Aβ plaques and vascular amyloid loads were detected, counted and measured as the percentage of total surface stained by monoclonal anti-Aβ antibody in the hippocampal and cortical sections. At the first experimental phase (preventive treatment), the amyloid plaque load observed in the representative photomicrographs (**Figure 2)** showed a significant difference across the four experimental groups (ANOVA, F=3.15, P<0.001).

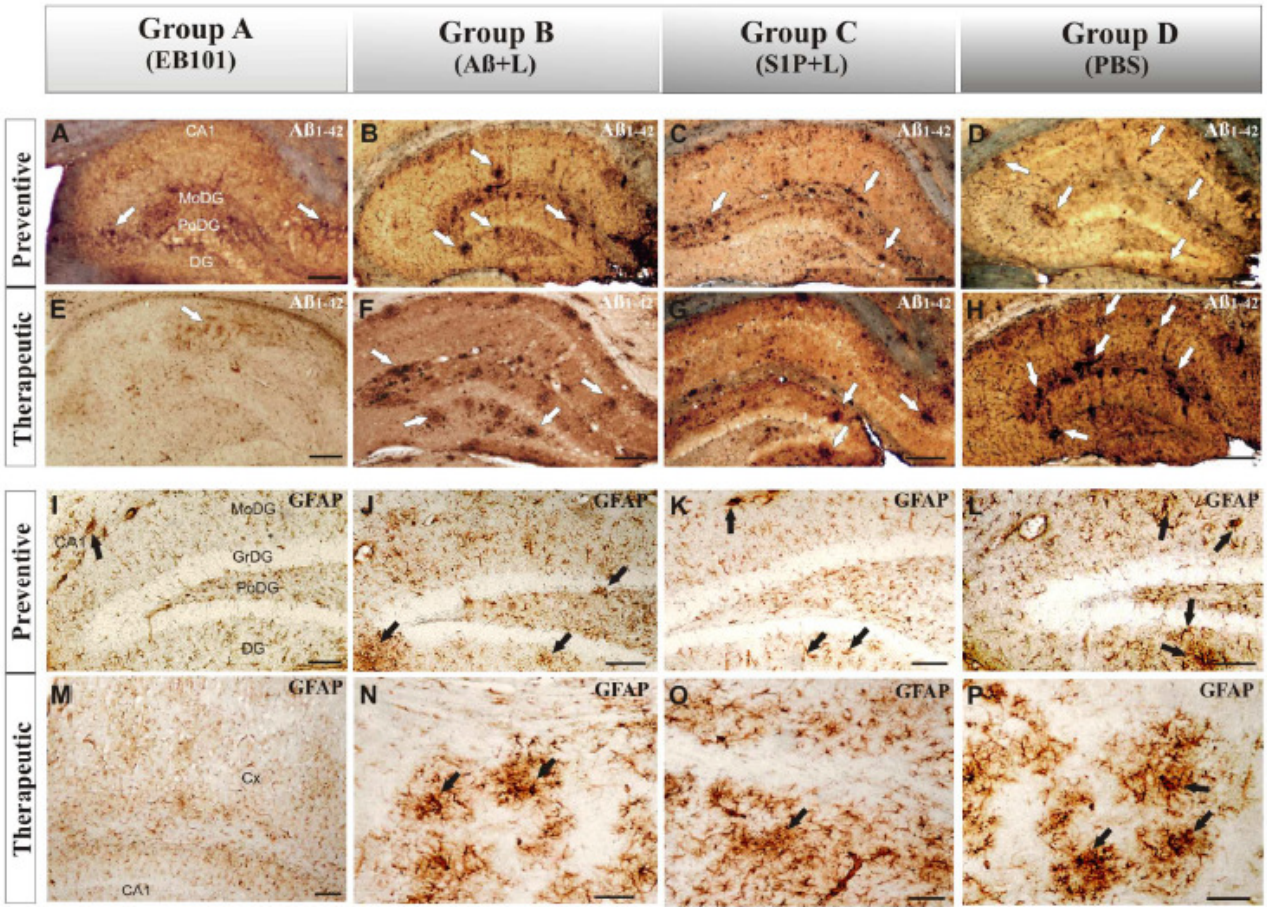

Figure 2. Effect of EB101 vaccine on beta amyloid (Aβ) deposits and astroglia in APP/PS1 mouse brain. Comparative photomicrographs of Aβ immunoreactivity were taken in the hippocampus (A-L) and cortical (M-P) brain regions of transgenic mice treated with the vaccine (Gr A) before Aβ plaques developed, preventive treatment (panels A-D, I-L) and after the Aβ plaques developed, therapeutic treatment (panels E-H, M-P). Scale bar: 100μm.

In this experiment we observed that EB101-immunized mice (**Group A, Figure 2A**) showed a significantly reduced hippocampal load when compared with the different vaccine components (**Groups B-C, Figure 2B, 2C**). In fact, the Aβ load in these different vaccine component groups (B-C) was not significantly different from PBS-treated mice (**Group D, Figure 2D**), although there was a correlation between the presence of S1P+Aβ in treatment regime and the total Aβ burden. The total Aβ plaque density and size quantification also supported this data, being performed by image analysis software of anti-Aβ antibody-stained sections in the hippocampus (**Figure 2A-2D**). Statistical analysis of the data obtained in the present study demonstrates that the mean Aβ plaque burden (**Figure 2A**) of group A (25 ± 5 p/s) was significantly different from the other treated groups (63 ± 6 p/s in 2B; 65 ± 6 p/s in 2C; 68 ± 6 p/s in 2D), which represents a notable decrease in the mean percentage of Aβ plaque area of 30.1-38.2% in the EB101-immunized mouse group relative to other tested groups. Therefore, these data show that in the preventive treatment period (**Figure 3**), the clearance effect of the EB101 vaccine on Aβ plaques per section was about 64% (**Figure 2A, Figure 3**) compared to positive controls, while 10% was detected in Aβ42/Liposome without S1P (B) and 7% in Liposome with S1P-treated mice (C).

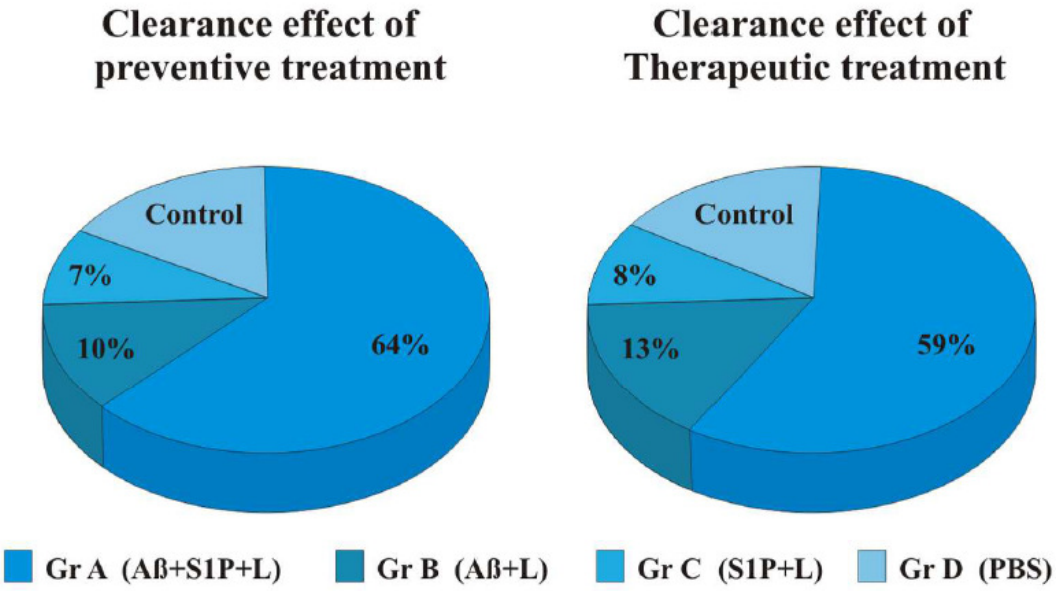

Figure 3. Comparative Aβ clearance effect. Pie charts representing the different effects of the treatment in the clearance of Aβ plaques in the transgenic mouse hippocampus. Both preventive (64%) and therapeutic (59%) treatment of EB101 showed a notable percentage of Aβ clearance compared to the other treatments in mouse brains.

Efficacy of S1P-containing niosomes (EB101) in clearing Aβ plaque burden

At the second experimental phase, (therapeutic treatment), we compared the effect of EB101 vaccine with different vaccine component groups in the reduction of Aβ in the brain after the plaques were established in the 35-week-old APP/PS1 transgenic mice (**Figure 2E-2H**). The results obtained showed that Aβ deposits were significantly reduced in the hippocampal brain sections of the EB101-treated mice (A), markedly different from the Aβ burden density observed in the different vaccine component groups (B-C). Photomicrographs of EB101-treated mice showed a few compacted Aβ plaques located at the CA1 layer of the hippocampus (**Figure 2E**), while in the other experimental groups no reduction in plaque density was observed (**Figure 2F-2H),** numerous large compacted Aβ plaques being located in almost every layer of each brain section. The data obtained showed that the treatment groups without Aβ1-42, C (**Figure 2G)** and D (**Figure 2H**), were the section most densely populated with Aβ plaques, while a slight reduction in this hallmark was observed in mouse group B (**Figure 2F).** As observed in the preventive treatment, the mean burden of Aβ plaques in group A (35 ± 4; **Figure 3**) was significantly different from the other treated groups (65 ± 5 in B; 70 ± 5 in C; 80 ± 6 in D), while the Aβ plaque area in the EB101-immunized mouse group was reduced by 29.7-34.5%, relative to the other groups tested (**Figure 3).** In both preventive and therapeutic treatments, a reduced Aβ plaque staining intensity was detected in the EB101-immunized mouse group, as shown in the photomicrographs of **figure**

2A,2E, contrasting with the intense Aβ-immunoreactivity of Aβ plaques in the other experimental groups.

Immune response effects of S1P-containing niosomes (EB101) in APP/PS1 mouse models

In order to analyze astrocyte activation in the hippocampal regions of APP/PS1 mice, which is known to be implicated in neuroinflammation, amyloidogenesis and neuronal cell death in AD, we used immunohistochemical analysis to quantify and compare the GFAP-reactive cells in the affected regions after each treatment period (**Figure 2I-P**). At the end of the preventive treatment (**Figure 2I-L**), EB101 significantly reduced the density of GFAP-reactive cell clusters in the hippocampal and cortical sections as compared with other treatments, such as Aβ/Lip without S1P (B), Lip with S1P (C) and PBS (D). Only a few scattered GFAP-reactive clusters, mainly at the CA1 hippocampal layers, were observed in the transverse section of the mouse brains treated with EB101 (**Figure 2I**), markedly contrasting with the numerous dystrophic reactive astrocytes observed in different hippocampal areas of mouse brains in groups B-D (**Figure 2J-2L**). After therapeutic treatment, mouse brains treated with EB101 were similar to those of control mice, mostly devoid of reactive GFAP clusters, except a few scattered ones in the CA1 hippocampal layers (**Figure 2M**). In contrast, there was a moderate density in group B (**Figure 2N**) and extensive density in group C (**Figure 2O**), and group D mice (**Figure 2P).** No astrocytosis was observed in control mice of each group during preventive or therapeutic treatment periods.

Table 1: Antibodies used for immunohistochemistry

Antibody	Antigen	Type	Source	Dilution	Ref.
β-amyloid	$A\beta_{1-42}$ (mouse)	Mouse monoclonal	Millipore	1:1000	5,6,38
Neurofibrillary tangles	NT (rabbit)	Rabbit polyclonal	Millipore	1:300	5,6,39
Glial fibrillary acidic p	GFAP (mouse)	Mouse monoclonal	Sigma	1:400	5,6,40

DISCUSSION

Liposome-based vaccines have demonstrated great effective potential in preventing and treating AD [7]. Based on previous effectiveness of active immunotherapy in reducing Aβ plaque accumulation [8], we have developed a new vaccine cocktail to circumvent the previous vaccine failures, due to an extensive T-cell-mediated immune response [9,10], by judiciously selecting an adjuvant that addresses all the above targets while avoiding the massive autoimmune activation of T-cells. This novel immunogen-adjuvant configuration consists of a physiological matrix, niosomes composed of naturally-occurring phospholipids (phosphatidylglycerol, phosphatidylcholine, and cholesterol), that has proven to be safe and efficacious in other types of vaccines. In order to increase the immunogenic potential we added a biologically active sphingolipid, sphingosine-1-phosphate (S1P) to this phospholipidic configuration. S1P is a phosphorylated product of sphingosine, catalyzed by sphingosine kinase, which has been reported to be an important lipid mediator acting both inside and outside the cell membrane [11,12]. Due to its role in the regulation of neuronal function, S1P facilitates glutamate secretion in hippocampal neurons by secretion actions, being involved in regulatory mechanisms of synaptic transmission. In particular, S1P itself causes glutamate secretion from presynaptic sites and potentiates glutamate-induced transmitter secretion in primary hippocampal neurons [13], possibly facilitating the formation of a positive activation cycle in excitatory neurons such as glutaminergic neurons. As extracellular effects, S1P binds to members of GTP-binding protein (G-protein)-coupled S1P receptor family (S1P1-5), triggering active immunity, angiogenesis, cell motility, and neurite growth [14,15]. As intracellular effects, S1P modulates cellular calcium mobilization, cell growth, and the inhibition of apoptosis [16,17].

Recently, the potential effect of S1P in neuronal regeneration was demonstrated in detail, since intracellular S1P enhances nerve growth factor-induced excitability in the sensory neurons of rats [18,19], controls migration of neuronal stem cells toward a site of spinal cord injury [20], and induces cytoskeletal rearrangements through small G-protein Rac activation [21], which seems to facilitate synaptic vesicle fusion to plasma membranes, enhancing transmitter secretion. Taking advantage of its important role in neuronal regeneration, cell growth, suppression of apoptosis and glutamate secretion from presynaptic sites that are affected in AD neuropathology, we incorporated the S1P into the phospholipid liposome to form a phospholipid-S1P-liposome matrix used as an immunogen-adjuvant to deliver the active antigen, Aβ1-42 [5]. Thus, this combination added a regenerative and anti-inflammatory component to the vaccine, key elements to increase neuronal activity and prevent inflammation in the brains of APPswe/PS1dE9 transgenic mice, improving the immunotherapeutic results previously reported by the studies based on Aβ-

immunization [22,23], while minimizing potential side effects.

EB101 vaccine was generated by using the hydration-rehydration method to efectively combine phospholipids, S1P and liposomes, forming niosomes against Aβ1-42 oligomers. This was used in other vaccines for an efficient liposomal-protein configuration, overcoming the immune pathological response encountered when using other types of adjuvants such as Freund's adjuvant [24,25], Quil-A [26], QS-21 and the detergent polysorbate 80 to solubilize Aβ, which are believed to induce a proinflammatory Th1 response [10,27]. Over the last decade, Aβ immunotherapy studies in APP-tg mice have reported different Aβ antibody levels, depending on the mouse model used, the immunization methodology, and the type of adjuvant for the vaccine formulation, emphasizing the significant impact of the adjuvants on the immune response evoked [28]. Considering this, we strategically planned a novel adjuvant-liposomal vaccine formulation in which the phospholipid-S1P-liposomes represent the pivotal structure that provides an inmunotherapeutic response markedly different from previous vaccine formulations tested in AD-like mice. Moreover, the significant effect of EB101, obtained in mice after the establishment of Aβ plaques in the hippocampus/neocortex and subsequent neuropathological changes, shows that EB101 vaccine presents a tremendous potential as a therapeutic agent to reverse neuropathological hallmarks in the AD-like brain, which represent an effective immunotherapeutic approach [29,5,6]. The results obtained in the preventive and therapeutic treatments showed that EB101 vaccine is efficacious not only in preventing the development of AD-like pathology but also in reducing it once established. The APPswe/PS1dE9 transgenic mice showed tiny and compact nonfibrillary amyloid plaque accumulation in the initial deposition period mainly at the hippocampus/neocortex, consistent with previous reported studies [30,31], whereas at later stages sparse Aβ-fibrillar plaques are progressively observed also at nearby cortical areas. After the preventive immunization period with EB101 vaccine, a significant reduction in density of Aβ plaque burden was observed in APP/PS1 transgenic mouse regions, as well as reduced burden areas when compared with APP/PS1 mice treated with different EB101 vaccine components or PBS. Schenk and colleagues [4] reported similar preventive immunization results in PDAPP mice, although differences in immunization efficiency and vaccine conformation have been improved in the present study. During this preventive period, EB101 immunization also prevented the progressive development of other AD pathological effects such as dystrophic plaque neurites, astrocytosis and motor coordination deficits. The hindrance of massive glial activation in response to early development of Aβ deposits has been accepted by the scientific community as the main key in efficient preventive immunization [32-35], this being the case of our EB101 vaccine, which has demonstrated, when compared with different EB101 vaccine components or PBS-treated mouse groups, that it prevents the development of astrocyte activation. Since the activation of astrocytes and microglia has been reported to induce the degradation of Aβ [36,37], the near-absence of Aβ-related astrocytosis in cortex and hippocampus of mice treated with EB101 vaccine confirmed the preventive effect against the development of AD-like pathology in this mouse model.

CONCLUSION

We have shown above the beneficial effect of combining the liposomal/sphingosine-1-phosphate adjuvant with an Aβ antigen (EB101 vaccine) to induce an effective immunological response with a wide anti-inflammatory spectrum in the APP/PS1 mouse model of AD. Based on the present results, the EB101 vaccine strongly prevented or reduced the main AD-like hallmarks (beta amyloid plaques and plaque-associated dystrophic neurites), induced the inhibition of astrocyte activation and neuroinflammatory response that naturally develops in APP/PS1 transgenic mice.

ACKNOWLEDGEMENTS

This work was supported by the III Research Grant Program of the Camilo José Cela University 2014-15 (TransBioModel – 2014/36).

REFERENCES

1. Tanzi R, Bertram L (2005) Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. Cell 120: 545-555.
2. Blennow K, De Leon M, Zetterberg H (2006) Alzheimer's disease. Lancet 368: 387-403.
3. Masliah E, Hansen L, Adame A, et al. (2005) Abeta vaccination effects on plaque pathology in the absence of encephalitis in Alzheimer disease. Neurology 64:129-131.
4. Schenk D, Barbour R, Dunn W, et al. (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 400:173-177.
5. Carrera I, Etcheverria I, Fernandez-Novoa L, et al. (2012) Vaccine development to treat Alzheimer´s disease neuropathology in APP/PS1 transgenic mice. Int J Alzheimers Dis 376138.
6. Carrera I, Etcheverría I, Li Y, et al. (2013) Immunocytochemical Characterization of Alzheimer Disease Hallmarks in APP/PS1 Transgenic Mice Treated with a New Anti-Amyloid- β Vaccine. Biomed Res Int 709145.
7. Davtyan H, Bacon A, Petrushina I, et al. (2014) Immunogenicity of DNA- and recombinant protein-

based Alzheimer Disease epitope vaccines. Hum Vaccin Immunother 13:10.

8. Rafii MS (2013) Update on Alzheimer's disease therapeutics. Rev Recent Clin Trials 2: 110-118.
9. Nicoll J, Wilkinson D, Holmes, et al. (2003) Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. Nat Med 9: 448-452.
10. Ferrer I, Boada M, Sánchez-Guerra, et al. (2004) Neuropathology and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease. Brain Pathol 14: 11-20.
11. Blaho VA, Hla T (2014) An update on the biology of sphingosine 1-phosphate receptors. J Lipid Res 55: 1596-1608
12. Nishi T, Kobayashi N, Hisano Y, et al. (2014) Molecular and physiological functions of sphingosine 1-phosphate transporters. Biochim Biophys Acta 5: 759-765.
13. Kajimoto T, Okada T, Yu H, et al. (2007) Involvement of sphingosine-1-phosphate in glutamate secretion in hippocampal neurons. Mol Cell Biol 9: 3429-40.
14. Gräler MH (2012) The role of sphingosine 1-phosphate in immunity and sepsis. Am J Clin Exp Immunol 2: 90-100.
15. Singh IN, Hall ED (2008) Multifaceted roles of sphingosine-1-phosphate: how does this bioactive sphingolipid fit with acute neurological injury? J Neurosci Res 7: 1419-1433.
16. Hopson KP, Truelove J, Chun J, et al. (2011) S1P activates store-operated calcium entry via receptor- and non-receptor-mediated pathways in vascular smooth muscle cells. Am J Phy Cell Physiol 4: 919-26.
17. Ratajczak MZ, Suszynska M, Borkowska S, et al. (2014) The role of sphingosine-1 phosphate and ceramide-1 phosphate in trafficking of normal stem cells and cancer cells. Expert Opin Ther Targets 1: 95-107.
18. Zhang YH, Vasko MR, Nicol GD (2006) Intracellular sphingosine 1-phosphate mediates the increased excitability produced by nerve growth factor in rat sensory neurons. J Physiol 1: 101-13.
19. Kays JS, Li C, Nicol GD (2012) Expression of sphingosine 1-phosphate receptors in the rat dorsal root ganglia and defined single isolated sensory neurons. Physiol Genomics 18: 889-901.
20. Kimura A, Ohmori T, Kashiwakura Y, et al. (2008) Antagonism of sphingosine 1-phosphate receptor-2 enhances migration of neural progenitor cells toward an area of brain. Stroke 12: 3411-7.
21. Biswas K, Yoshioka K, Asanuma K, et al. (2013) Essential role of class II phosphatidylinositol-3-kinase-C2α in sphingosine 1-phosphate receptor-1-mediated signaling and migration in endothelial cells. J Biol Chem 4: 2325-39.
22. Davtyan H, Petrushina I, Ghochikyan A (2014) Immunotherapy for Alzheimer's Disease: DNA- and Protein-Based Epitope Vaccines. Methods Mol Biol 1143: 259-81.
23. Wisniewski T, Goñi F (2014) Immunotherapy for Alzheimer's disease. BiochemPharmacol 4: 499-507.
24. Schenk D, Barbour R, Dunn W, et al. (1999) Immunization with amyloid-β attenuates Alzheimer disease-like pathology in the PDAPP mouse. Nature 400: 173-177.
25. Wilcock DM, Gharkholonarehe N, Van Nostrand WE, et al. (2009) Amyloid reduction by amyloid-β vaccination also reduces mouse tau pathology and protects from neuron loss in two mouse models of Alzheimer's disease. J Neurosci 29: 7957-7965.
26. Ghochikyan A, Mkrtichyan M, Petrushina I, et al. (2006) Prototype Alzheimer's disease epitope vaccine induced strong Th2-type anti-Abeta antibody response with Alum to Quil A adjuvant switch. Vaccine 13: 2275-82.
27. Panza F, Frisardi V, Solfrizzi V, et al. (2012) Immunotherapy for Alzheimer's disease: from anti-β-amyloid to tau-based immunization strategies. Immunotherapy 2: 213-38.
28. Mount A, Koernig S, Silva A, et al. (2013) Combination of adjuvants: the future of vaccine design. Expert Rev Vaccines 7: 733-46.
29. Lannfelt L, Relkin NR, Siemers ER (2014) Amyloid-ß-directed immunotherapy for Alzheimer's disease. J Intern Med 3: 284-95.
30. Trinchese F, Liu S, Battaglia F, et al. (2004) Progressive age-related development of Alzheimer-like pathology in APP/PS1 mice. Ann Neurol 55: 801-814.
31. Jankowsky J, Fadale D, Anderson et al. (2003) Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase. Hum Mol Genet 13: 159-170.

32. Davtyan H, Ghochikyan A, Petrushina I, et al. (2013) Immunogenicity, efficacy, safety, and mechanism of action of epitope vaccine (Lu AF20513) for Alzheimer's disease: prelude to a clinical trial. J Neurosci 11: 4923-34.

33. Zotova E, Holmes C, Johnston D, et al. (2011) Microglial alterations in human Alzheimer's disease following Aβ42 immunization. Neuropathol Appl Neurobiol 5: 513-24.

34. Cacabelos R, Martínez R., Fernández-Novoa L, et al. (2012) Genomics of Dementia: APOE- and CYP2D6-Related Pharmacogenetics. Int J Alzheimers Dis 518901.

35. Cacabelos R, Martínez-Bouza R (2011) Genomics and pharmacogenomics of dementia. CNS Neurosci Ther 17: 566-76.

36. Morales I, Guzmán-Martínez L, Cerda-Troncoso C, et al. (2014) Neuroinflammation in the pathogenesis of Alzheimer's disease. A rational framework for the search of novel therapeutic approaches. Front Cell Neurosci 8: 112.

37. Birch AM, Katsouri L, Sastre M (2014) Modulation of inflammation in transgenic models of Alzheimer's disease. J Neuroinflammation 11: 25.

38. Schroeter S, Khan K, Barbour R, et al. (2008) Immunotherapy reduces vascular amyloid-beta in PDAPP mice. J Neurosci 28: 6787-6793.

39. Suo Z, Cox A, Bartelli N, et al. (2007) GRK5 deficiency leads to early Alzheimer-like pathology and working memory impairment. Neurobiol Aging 28: 1873-1888.

40. Morgan D (2006) Modulation of microglial activation state following passive immunization in amyloid depositing transgenic mice. Neurochem Int 49: 190-194.