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Amyloid beta-HSP60 peptide conjugate vaccine treats a mouse model of Alzheimer's disease

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ABSTRACT

Active vaccination with amyloid beta peptide (Aβ) to induce beneficial antibodies was found to be effective in mouse models of Alzheimer's disease (AD), but human vaccination trials led to adverse effects, apparently caused by exuberant T-cell reactivity. Here, we sought to develop a safer active vaccine for AD with reduced T-cell activation. We treated a mouse model of AD carrying the HLA-DR DRB1*1501 allele, with the Aβ B-cell epitope (Aβ 1–15) conjugated to the self-HSP60 peptide p458. Immunization with the conjugate led to the induction of Aβ-specific antibodies associated with a significant reduction of cerebral amyloid burden and of the accompanying inflammatory response in the brain; only a mild T-cell response specific to the HSP peptide but not to the Aβ peptide was found. This type of vaccination, evoking a gradual increase in antibody titers accompanied by a mild T-cell response is likely due to the unique adjuvant and T-cell stimulating properties of the self-HSP peptide used in the conjugate and might provide a safer approach to effective AD vaccination.

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1. Introduction

Alzheimer's disease (AD) is a progressive, degenerative disorder of the brain and the most common cause of dementia among the elderly. Aβ1–40 and Aβ1–42 peptides are generated from the cleavage of amyloid precursor protein (APP) by beta- and gamma-secretases and are the major components of senile plaques and Aβ fibrils observed in brains of AD patients [1–4]. They are considered to play a crucial role in the pathogenesis of AD [5] and thus serve as a target for therapeutic approaches aimed at decreasing their production, deposition and toxicity.

Among the different Aβ-targeted approaches to AD treatment, Aβ immunotherapy has been shown to induce a marked reduction in amyloid burden and an improvement in cognitive functions in different animal models [6–13]. Although preclinical studies had been successful, the initial human clinical trial of an active Aβ vaccine (AN-1792 trial performed by Elan Pharmaceutical) was stopped due to the development of meningoencephalitis in approximately 6% of the vaccinated AD patients [14]. These severe side effects were attributed to the use of QS21, a very strong adjuvant, and the full length of the Aβ peptide, the combination of which might have led to the development of pathogenic T-cells [15,16]. Nevertheless, some encouraging outcomes, including signs of cognitive stabilization and apparent plaque clearance, were found in a subset of patients who generated specific antibodies upon Aβ immunization. These promising results have motivated further efforts to refine Aβ immunotherapy to produce effective and safer active and passive vaccines for AD.

While passive immunization against Aβ has shown to be effective in mouse models of AD (currently being tested in a phase III clinical trial), the segregation between the B-cell and T-cell epitopes within Aβ1–42 [9,10,17,18] may allow for the development of safer active vaccines. Aβ T-cell epitopes are located primarily between residues 10 and 42 of Aβ in mice [9,10,17,18] and in humans [19,20], and thus the N-terminal portion of Aβ has been used to generate active Aβ vaccines. The N-terminal portion of Aβ1–15 conjugated to the T-cell epitope of bovine serum albumin [18,21], or a promiscuous foreign T-cell epitope PADRE [22] was shown to elicit effective antibody responses without stimulating an Aβ-specific T-cell response. These vaccination studies have led to preclinical studies using the N-terminal portion of Aβ presented on the surface of virus particles [23], liposomes [24], or administered as Aβ coding DNA plasmids or viral vectors [25–28].

Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; CFA, complete Freund's adjuvant; HSP, heat shock protein; HLA, human leukocyte antigen; IFA, incomplete Freund's adjuvant; IFN-γ, interferon-gamma; IL, interleukin; IHC, immunohistochemistry; Ig, immunoglobulin; LN, lymph node; MHC, major histocompatibility complex; PDGF, platelet-derived growth factor; TCR, T-cell receptor; TLR, Toll-like Receptor.

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In addition, a phase II clinical trial ACC-001, using the N-terminal of Aβ conjugated to diphtheria toxin is being carried out by Wyeth.

Among the variety of existing carriers that can provide support for antibody production when conjugated to the N-terminal portion of Aβ, p458, a 17 amino acid peptide derived from the 60-kDa heat shock protein (HSP60), has unique properties. First, the full length HSP60 protein is recognized by the immune system and plays an important role in the regulation of immunity, autoimmunity and inflammation [29–31]. Second, it has been previously established that when complexed with an antigen, HSP60, like other heat shock proteins, acts as an adjuvant, promoting both humoral and cellular immune responses [32,33]. These adjuvant properties are attributed to the ability of this protein to activate dendritic cells and macrophages via TLR pathways [34]. p458 has thus been efficiently used as a carrier to initiate antibody production in a number of bacterial [32–35] and viral vaccines [36,37].

In the current study, we used the p458 peptide as a carrier conjugated to Aβ1–15 to promote Aβ-specific antibody production. We demonstrate that vaccination of APP Tg bearing the DRB1*1501 allele with Aβ1–15 conjugated to p458 resulted in Aβ-specific antibody production, associated with only very mild HSP60-specific T-cell activation, Aβ plaque clearance and a decrease in the level of microglial activation in the brain.

2. Materials and methods

2.1. Antigens

We designed a novel peptide by fusing the Aβ1–15 region with HSP60 p458. The Aβ1–15 region contains the Aβ B-cell epitope, whereas p458 provides T-cell support for antibody production. The whole sequence of the conjugate was DAEFRHDSGYEVHHQNEDQKIGIEIIKRTLKI, referred to hereafter as Aβ–HSP60. Aβ–HSP60, p458 and Aβ1–42 (the 42 residues of Aβ) peptides used in the study were synthesized by GenScript Corp. (Piscataway, NJ).

2.2. Mice

APP Tg mice (J20 line [13]) on a C57BL6 background expressing the human mutated $APP_{Sw,Ind}$ under the PDGF promoter were kindly donated by L. Mucke. Tg mice co-expressing HLA-DR DRB1-1501 (DR15 mice [38]) and a human T-cell receptor (TCR) specific for MBP 85-99 on a C57BL6 background were kindly donated by Vijay K. Kuchroo and Daniel M. Altmann. DR15 mice were crossed with APP Tg mice to generate APP/DR15 double-Tg mice. These mice all develop Alzheimer-like disease spontaneously. Mice were kept and bred at the animal facility of Ben-Gurion University, Beer Sheva, Israel, in autoclaved cages with autoclaved bedding, food and water. All surgical and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Ben-Gurion University of the Negev.

2.3. Vaccination

Mice were vaccinated by subcutaneous injection with Aβ–HSP60, p458 or Aβ1–42 (100 μg/mouse) emulsified in complete Freund's Adjuvant (CFA) or incomplete Freund's Adjuvant (IFA) (Sigma, Israel) and at the intervals indicated in figure legends.

2.4. Cytokine ELISA

Lymph node (LN)-derived cells were cultured (10×10^6 cells/mL) in U-shaped 96-well-plate culture dishes in Biotarget serum-free medium (Biological Industries, Israel) containing 1% Pen/Strep. For IL-2 and IL-4 measurements, supernatants were collected 24 h after cell seeding. For IFN-γ and IL-17A measurements, supernatants were collected 48 and 72 h after cell seeding, respectively. Sandwich ELISA was implemented for measuring cytokine concentrations in the supernatants, according to manufacturer's instructions (Biolegend, San Diego, CA).

2.5. Antibody ELISA

Serum samples were subjected to ELISA using the goat anti-mouse Ig and goat antimouse IgG–HRP conjugated antibodies (Southern Biotech, Birmingham, AL). Briefly, plates were coated with Aβ1–42 (3 μg/mL) or goat anti mouse Ig (0.2 μg/mL) (Southern Biotech, Birmingham, AL) for samples or standards, respectively. After blocking, the standards and the samples were applied. Standards [purified mouse IgG (Southern Biotech, Birmingham, AL)] were used at 20, 15, 10, 7.5, 5, 2.5, 1 and 0 ng/mL. Samples were applied at different series of dilutions in the range of 1:1000–1:100,000. The goat antimouse IgG–HRP was used at 0.01 μg/mL. Standards, samples and secondary antibody were diluted with blocking solution (1% BSA in PBS).

The antibody isotypes were tested using Mouse Immunoglobulin Isotype Panel kit (Southern Biotech, Birmingham, AL) according to manufacturer's instructions. To analyze antibody binding to Aβ–HSP60 or p 458 peptides, plates were coated with these peptides (3 μg/mL).

2.6. Immunohistochemistry (IHC)

Mice were killed with an overdose of isofluorane, and their brains were rapidly excised and fixed in OCT (Tissue-Tek, Torrance, CA). The tissues were frozen in isopentane (cooled in liquid nitrogen) and stored at −80 °C. Sagittal sections (12 μm thick) were taken throughout the hippocampus and fixed in ice-cold methanol for 2 min, then in 4% formaldehyde for 4 min, and then washed with distilled water and phosphate-buffered saline (PBS)/Tween (0.05%). Prior to staining, primary antibody diluting buffer (Biomeda Corp., Foster City, CA) was used to block nonspecific binding. Anti-CD11b (Serotec, Raleigh, NC) was diluted 1:25. Rabbit anti-human Aβ antibodies were generated at the animal facility of Ben-Gurion University, Beer Sheva, Israel, and were diluted 1:500. All secondary antibodies were conjugated to Alexa-488, Alexa-546, or Alexa-647 (Invitrogen, Carlsbad, CA) and diluted 1:500. TO-PRO 3 (Molecular Probes, Invitrogen, USA) was used for nuclei staining at a dilution of 1:3000. Sections were examined under an Olympus Fluoview FV1000 confocal laser scanning microscope.

2.7. Confocal imaging analysis

Quantification analysis of Aβ plaques and $CD11b^{high}$ cells in the brain was performed in four sections (12 μm thick) per hemisphere stained for Aβ and CD11b, for accurate representation of the hippocampus area. Fluorescence intensity was first obtained in sections from control mice (immunized with adjuvant only), and identical laser-scanning parameters were then used for the entire experiment. Using Volocity 3D image analysis software (Improvision, Waltham, MA), an intensity threshold was set to mark only those areas showing significant staining as previously described [39]. The average fluorescence area per brain section was calculated for each of the analyzed groups.

2.8. Statistical analysis

All statistical analyses were performed using GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego, CA). All variables are expressed as mean ± SD or SEM as indicated in figure

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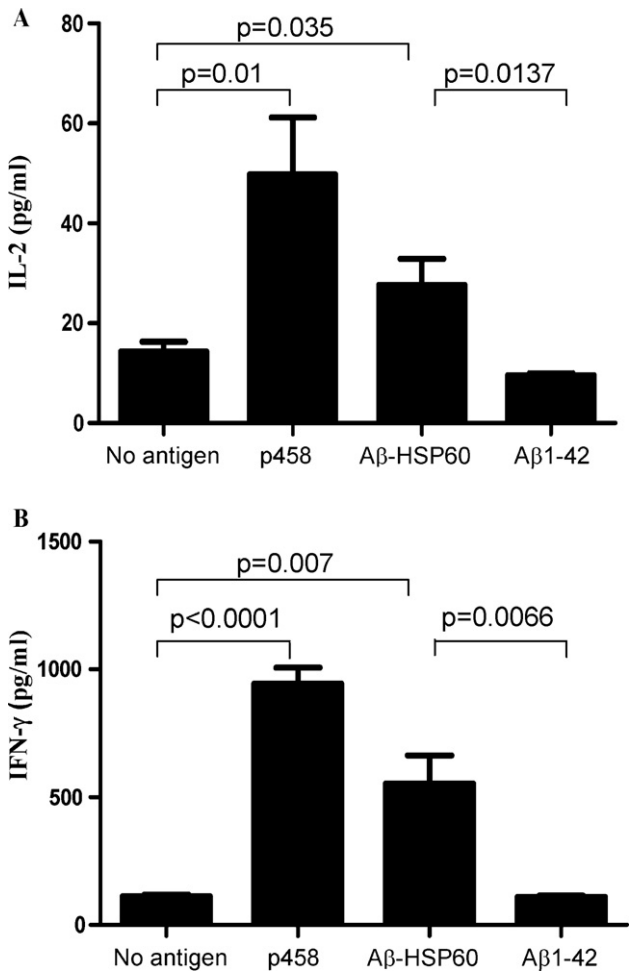

Fig. 1. Cytokine production following Aβ–HSP60 short-term immunization of C57BL6 mice. Mice aged 2 months were immunized with Aβ–HSP60 emulsified in CFA as described in Section 2. Ten days after immunization LN-derived cells were pooled and stimulated with Aβ–HSP60, p458 or Aβ1–42 antigens. IL-2 (A) and IFN-γ (B) production in supernatants were measured by ELISA. The bars represent the mean ± SEM for pooled LN-derived cells within one experiment ($n = 3$) out of two independent repeats performed ($n = 6$). p-Values were calculated by unpaired two-tailed t-test.

legends. p values were calculated using unpaired two-tailed t-test for the entire study except for Figs. 2 and 4A where serum samples of individual mice were analyzed before and after vaccination and thus a paired one-tailed t-test was performed.

3. Results

3.1. Aβ–HSP60 vaccination induces HSP60-specific T-cell responses in C57BL6 mice

To characterize the immunogenicity of Aβ–HSP60, we initially characterized the T-cell response to Aβ–HSP60 vaccination in young C57BL6 wild-type mice. For this purpose, 2-month-old C57BL6 mice ($H2^b$ MHC class II haplotype) were vaccinated with Aβ–HSP60 emulsified in CFA. Ten days later the mice were killed and their popliteal lymph nodes were excised. LN-derived cells were then cultured and stimulated with increasing concentrations of Aβ–HSP60, Aβ1–42 or p458. T-cell-dependent cytokine production (IL-2 and IFN-γ; IL-4 and IL-10; and IL-17A, primarily produced upon activation of Th1; Th2; and Th17 T-cells, respectively) was analyzed by ELISA. IL-2 (Fig. 1A) and IFN-γ (Fig. 1B), measured 24 and 48 h after stimulation, respectively, were increased in the supernatants upon p458 and Aβ–HSP60, but not Aβ1–42 stimulation, in a dose-dependent manner. No IL-4, IL-10 or IL-17A production was detected following immunization with Aβ–HSP60 (data not shown).

3.2. Aβ–HSP60 vaccination induces Aβ-specific antibody production in C57BL6 mice

In light of the fact that the T-cell response to Aβ–HSP60 immunization was very mild, we sought to determine whether such T-cell activation could provide sufficient support for Aβ-specific antibody production. To this end, 2-month-old wild type C57BL6 mice were vaccinated four times with the Aβ–HSP60 peptide emulsified in IFA at 2-week intervals. The mice were bled two weeks after each immunization and antibody production was determined in the sera by ELISA as described in Section 2. Limited titers of Aβ-specific antibodies were detected after the first immunization (3.14 ± 2.47 μg/mL), raised subsequently with the following injections and reached 7.8 ± 4.015, 46.9 ± 30.8 and 119.9 ± 43.6 μg/mL after the second, third and fourth vaccinations, respectively (Fig. 2A). The antibodies recognized both the entire Aβ1–42 and Aβ–HSP60 peptides, and no p458-specific antibodies were detected (Fig. 2B). The pattern of antibody isotypes evoked upon Aβ–HSP60 immunization revealed that IgG1, IgG2b and IgM were the predominant isotypes with relatively lower titers of IgG2c (Fig. 2C).

3.3. Aβ–HSP60 vaccination promotes a milder T-cell response than Aβ1–42 in Tg mice carrying the HLA-DR allele DRB1*1501

We have recently demonstrated that humans carrying the highly frequent HLA-DR DRB1*1501 allele have Aβ specific T cells in their circulation. Aβ1–42 was also highly immunogenic in humanized mice carrying this allele (DR15 Tg mice) [20]. In both human individuals and mice carrying the DRB1*1501 allele, Aβ28–42 was the dominant T-cell epitope [20]. We thus sought to determine the immune response evoked by Aβ–HSP60 (where the Aβ28–42 T-cell epitope was replaced by p458) in young DR15 Tg mice compared with that evoked by Aβ1–42. Two-month-old DR15 Tg mice were vaccinated with Aβ–HSP60 or Aβ1–42 following the same protocol used for C57BL6 mice. The mice were killed 10 days after the immunization and their popliteal draining LNs were excised. LN-derived cells were then analyzed for cytokine secretion. In LN-derived cultures from both Aβ–HSP60 (white bars) and Aβ1–42 (black bars) immunized mice, the cytokines IL-2 (Fig. 3A), IFN-γ (Fig. 3B) and IL-17A (Fig. 3C) were increased in the supernatant upon activation with the respective antigen, in a dose dependent manner. Whereas remarkably high amounts of IL-2 (Fig. 3A), IFN-γ (Fig. 3B) and IL-17A (Fig. 3C) were detected in cultures from mice vaccinated with Aβ1–42, their presence was minor in cultures derived from Aβ–HSP60-vaccinated mice (Fig. 3A–C). Taken together these data indicate that the Aβ–HSP60 peptide conjugate evokes a significantly milder T-cell response than does Aβ1–42 in DR15 mice.

3.4. The B-cell response elicited in Aβ–HSP60-vaccinated DR15 Tg mice

To determine Aβ antibody production during a long-term immunization protocol, 2-month-old DR15 Tg mice were immunized four times with Aβ–HSP60 or Aβ1–42 emulsified in IFA at 2-week intervals as described in Section 2. Two weeks after each immunization, the mice were bled and their sera were analyzed for antibody production. As shown for immunized C57BL6 mice (Fig. 2), Aβ–HSP60 immunization of DR15 Tg mice promoted the gradual increase of Aβ1–42-specific antibody titers at the fol-

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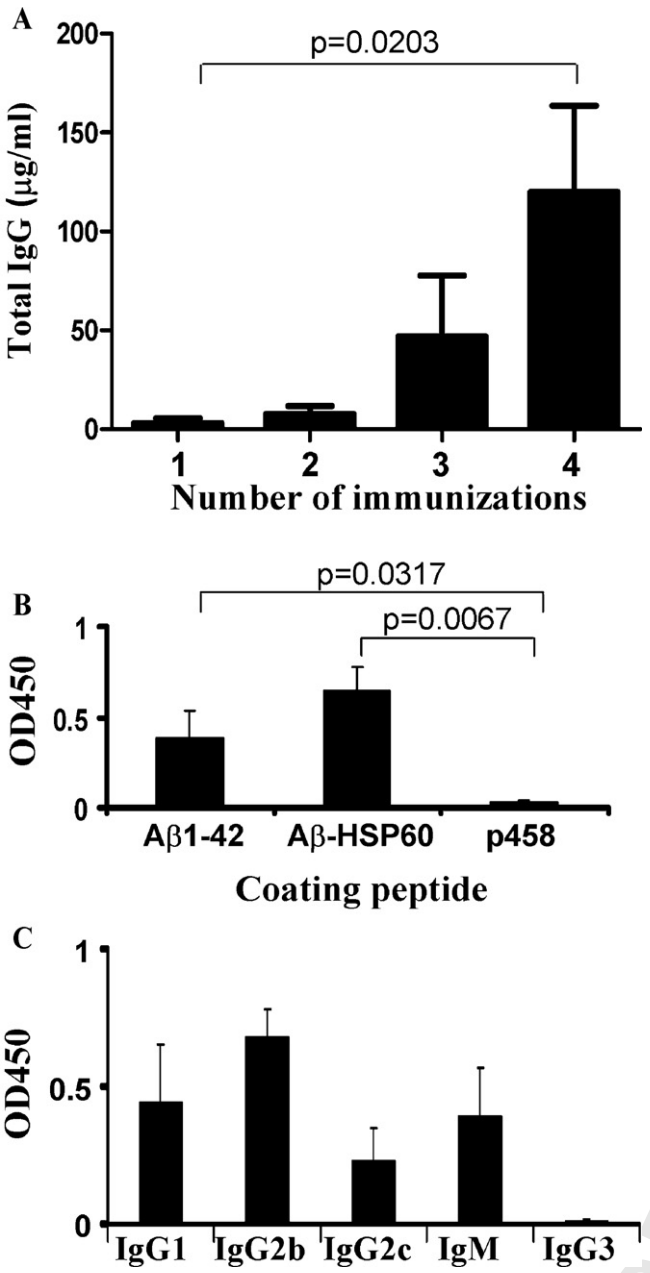

Fig. 2. Kinetics, specificity and isotypes of antibodies evoked by Aβ–HSP60 vaccination in C57BL6 mice. C57BL6 mice ($n = 6$) aged two months were immunized four times with Aβ–HSP60 emulsified in IFA at 2-week intervals. Mice were bled before vaccination and two weeks after each immunization, and antibodies were analyzed for titers (A). Specificity (B) and isotypes (C) were analyzed after the last boost by ELISA as described in Section 2. The bars represent the mean ± SEM. *p*-Values were calculated by paired one-tailed *t*-test. The data show two independent repeats of the experiment.

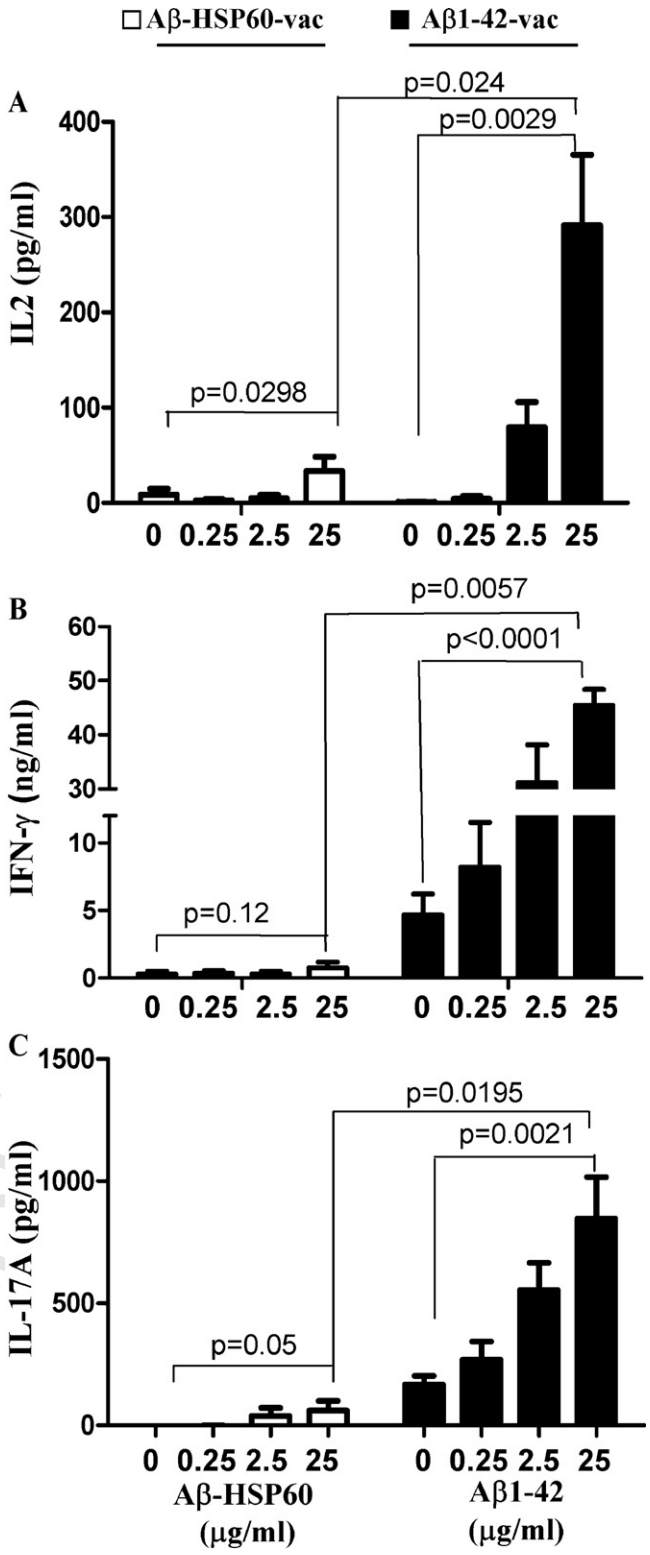

Fig. 3. Comparison of cytokine production following Aβ–HSP60 and Aβ1–42 vaccination in DR15-Tg mice. DR15 mice aged two months were immunized with Aβ–HSP60 ($n = 7$) or Aβ1–42 ($n = 9$) emulsified in CFA. Ten days later mice were killed, and LN-derived cells were stimulated with increasing concentrations of Aβ–HSP60 (white bars) or Aβ1–42 (black bars) for Aβ–HSP60 and Aβ1–42-vaccinated mice, respectively. The cytokines IL-2 (A), IFN-γ (B) and IL-17A (C) were measured by ELISA. The data show one representative experiment out of two independent repeats performed. The bars represent the mean value obtained for each of the antigen concentrations for Aβ–HSP60 vaccination ($n = 4$) and for Aβ1–42 vaccination ($n = 6$) ± SEM. *p*-Values were calculated by unpaired two-tailed *t*-test.

lowing injections of Aβ–HSP60 up to a maximal concentration of 144 ± 78 μg/mL after the third immunization (Fig. 4A). Immunization with either Aβ–HSP60 or Aβ1–42 evoked antibodies specific to Aβ1–42 and Aβ–HSP60 but not to p458 (Fig. 4B). The antibody titer evoked upon Aβ–HSP60 vaccination was significantly lower than after Aβ1–42 vaccination, but the pattern of antibody isotypes was similar. IgG1 and IgG2b were found to be the predominant isotypes with relatively low levels of IgG2c and IgM following immunization with either Aβ–HSP60 or Aβ1–42 (Fig. 4C). Since HSP was shown to have adjuvant properties [34,35] we examined whether the Aβ–HSP60 conjugate alone evokes an immune response upon intracutaneous injection. Whereas significant T-cell

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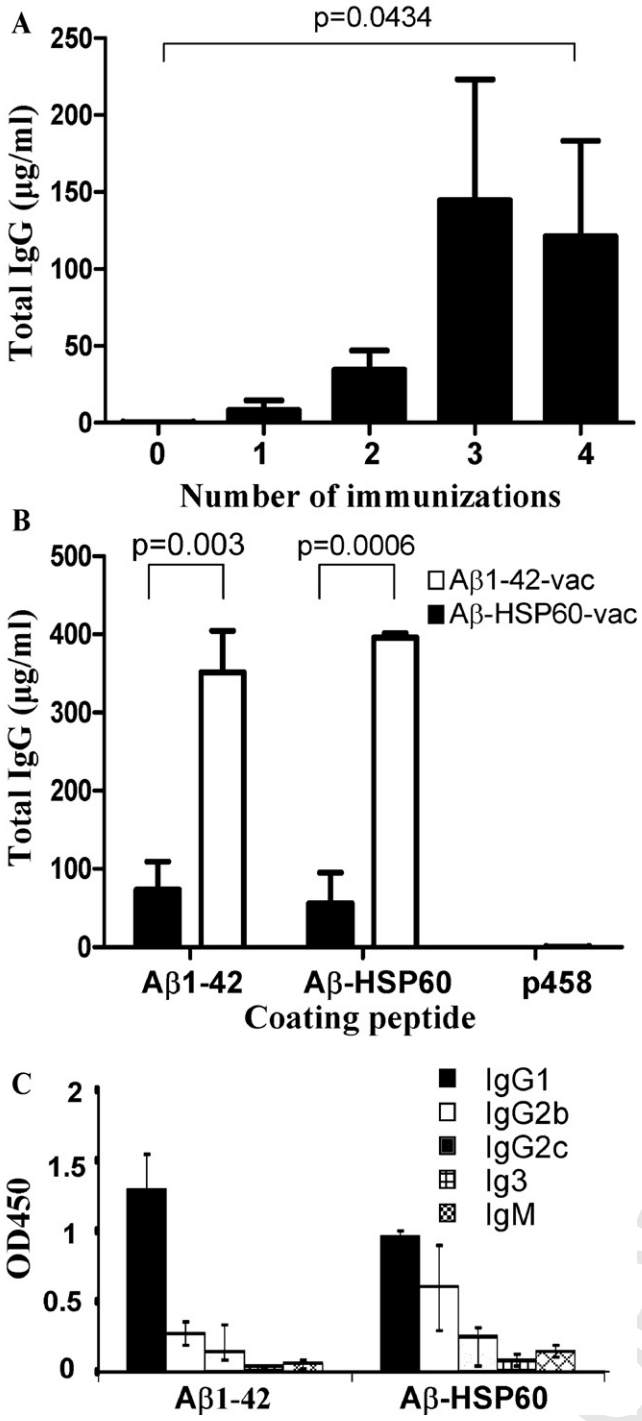

Fig. 4. Antibody characteristics in Aβ–HSP60 and Aβ1–42-vaccinated DR15 mice. DR15-Tg mice aged two months were immunized four times with Aβ–HSP60 ($n = 6$) or Aβ1–42 ($n = 3$) emulsified in IFA at 2-week intervals. Mice were bled two weeks after each immunization. Total antibody titers were measured following each immunization with Aβ–HSP60 (A). The bars represent the mean antibody levels obtained in each group ± SEM. Total IgG antibody titers and specificity (B) and antibody isotypes (C) were compared between the two groups (vaccinated with Aβ–HSP60 or Aβ1–42), two weeks after the last immunization. The bars represent the mean antibody levels obtained in each group ± SEM. *p*-Values were calculated by paired one-tailed *t*-test for the data shown in panel A and unpaired two-tailed *t*-test for the data shown in panel B.

responses above background levels were not detected (data not shown), we demonstrate that following 4 intracutaneous injections low levels of Aβ antibodies were produced (Supplementary Fig. 2).

3.5. Aβ–HSP60 immunization of APP/DR15 Tg mice promotes the clearance of amyloid plaques

To determine whether Aβ–HSP60 immunization reduces the deposition of Aβ in the brain, 5.5-month-old APP/DR15 Tg mice were immunized five times with Aβ–HSP60 emulsified in IFA at 25 day intervals. Control mice were injected with PBS emulsified in IFA. Mice were killed 25 days after the last immunization and serum samples were tested for Aβ antibodies by ELISA. As shown in Fig. 5A, Aβ–HSP60 immunization evoked the gradual production of Aβ1–42-specific antibodies. No Aβ-specific antibodies were observed in the control group (data not shown). Brain sections were then immunostained with Aβ antibodies and amyloid deposits were quantified as described in Section 2. Representative images of brain sections show remarkably fewer Aβ deposits (green) in the hippocampal dentate gyrus area of Aβ–HSP60-vaccinated mice relative to control mice immunized with adjuvant alone (Fig. 5B). Quantification of Aβ deposition in the brain revealed a significant reduction in the brain area occupied by Aβ in Aβ–HSP60-immunized mice compared with the IFA-immunized control group (Fig. 5C).

To characterize the extent of microglial accumulation and activation associated with Aβ clearance, brain sections were immunostained with Aβ and CD11b antibodies. Co-staining of Aβ (red) and CD11b (green) shows co-localization between Aβ plaques and activated microglia in the hippocampi of both Aβ–HSP60-immunized and control mice (Fig. 6). Importantly, the significant decrease in Aβ plaques in the Aβ–HSP60-vaccinated group was accompanied by a proportional decrease in the amount of activated microglia detected in these areas of the brain (Fig. 6).

Recently we have shown that limited expression of IFN-γ in the brains of APP Tg mice is sufficient to promote CD4 T-cell migration to the brain upon vaccination with Aβ1–42 [39,42]. We thus sought to use this mouse model to examine whether p458 itself affects the deposition of Aβ in the brain. As shown in Supplementary Fig. 1A, Aβ antibodies were produced following Aβ–HSP60 but not p458 vaccination. Concomitantly, significant clearance of Aβ was observed in mice vaccinated with Aβ–HSP60 but not in mice vaccinated with p458 (Supplementary Fig. 1B and C).

4. Discussion

In this study, the N-terminal region Aβ1–15, which contains most of the B-cell epitopes of Aβ-peptide, was conjugated to the HSP60 peptide p458 to generate a novel AD vaccine. We first characterized the immune response towards the Aβ–HSP60 conjugate in C57BL6 mice carrying the $H2^b$ MHC class II haplotype. Our results demonstrated a very mild T-cell response specific to the HSP60 peptide, associated with a gradual increase in specific antibodies to the Aβ1–42 peptide. Immunization of mice carrying the human HLA-DR DRB1*1501 allele with Aβ–HSP60 resembled the humoral response elicited by Aβ1–42 by means of antibody specificity and isotypes, however, with a substantially milder antibody and T-cell response. Furthermore, immunization of APP/DR15 double transgenic mice with Aβ–HSP60 resulted in significant Aβ plaque clearance from the brain associated with a decrease in the inflammatory response in the brain.

The utilization of Aβ1–42 for active vaccination of AD patients (Elan's clinical study) caused a pathogenic activation of the immune system against Aβ within the CNS [14]. It thus became clear that the immune response evoked to Aβ should be well controlled for its

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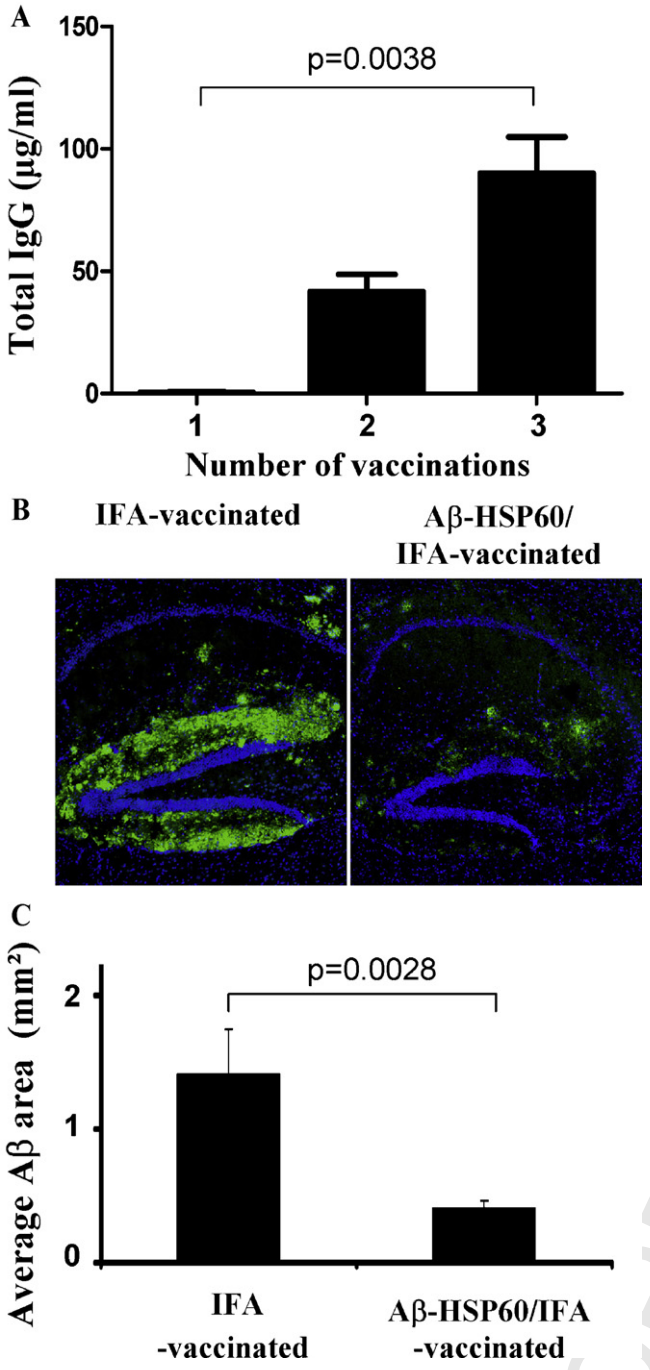

Fig. 5. Aβ1–42-specific antibody production and Aβ plaque clearance from the brains of APP/DR15 Tg mice immunized with Aβ–HSP60. APP/DR15 Tg mice ($n = 4$) aged 5.5 months were immunized five times with Aβ–HSP60 emulsified in IFA at 25 day intervals. Control mice ($n = 3$) were immunized with PBS emulsified in IFA. Twenty five days after the last immunization the mice (aged 9.5 months) were killed and their brains were excised and analyzed for Aβ1–42-specific antibody production and Aβ plaque deposition as described in Section 2. (A) Gradual production of Aβ1–42-specific antibody during immunization. Bars represent mean antibody titers ± SEM. p-Values were calculated by unpaired two-tailed t-test. (B) Brain sections were immunolabeled with anti-Aβ (green) and nuclei were stained with TO-PRO 3 (blue). Representative sections from Aβ–HSP60-vaccinated and adjuvant-only vaccinated groups are shown. (C) The average sum of Aβ-stained area was quantified for each 12-μm-thick section using the Volocity 3S Image Analysis software, as described in Section 2. The bars represent mean ± SEM. p-Value was calculated by unpaired two-tailed t-test. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

effector function in the brain, a feature which can be achieved by the choice of adjuvant [22], route and dose of the vaccine [40], and alterations of the T-cell epitopes. Utilizing the fact that the B-cell and T-cell epitopes are segregated in the Aβ1–42 peptide [19,20,41,42], numerous active vaccination approaches conjugating the Aβ1–15 region to various carriers have been established (see review [27]). The non-self carriers in these vaccines indeed prevented the T-cell response against Aβ; however, they evoked a strong T-cell response against the foreign epitopes and high titers of Aβ-specific antibodies generated in a relatively short period of time. Compared to the T-cell responses induced by previous attempts at Aβ1–42 immunization, our results show that the Aβ–HSP60 vaccine promoted a very mild T-cell response, evident by the significantly lower production of the proinflammatory cytokines IFN-γ and IL-17A in lymphocyte cultures from DR15 mice. Notably, the mild T-cell response induced by Aβ–HSP60 caused a gradual increase in specific Aβ antibody titers, which were sufficient for effective clearance of Aβ plaques from the brain of aged APP-Tg mice. Importantly, we demonstrate that in APP/IFN-γ mice where Aβ antibody titers were even lower, presumably as a result of the lower immunogenicity of p458 in this background, robust plaque clearance still occurred following vaccination with Aβ–HSP60.

In line with our study, which demonstrates no pathogenic reaction to Aβ–HSP60 immunization, numerous studies have established the unique immunogenic characteristics of mammalian HSPs-derived peptides [32,33,43–46]. In addition, no induction of pathogenic autoimmunity was observed when p458 was injected into mice alone [47], as a conjugate with the pneumococcal capsular polysaccharide [34], or as a chimeric peptide with a West Nile virus-derived peptide [36], suggesting that the activation of HSP60-specific T cells is tightly regulated. While there are still open questions regarding these unique properties of HSP60-specific T cells i.e., how these T cells are selected in the thymus to be part of the T-cell repertoire and how they are stimulated to play a differential role in immune activation and regulation, it seems reasonable to suggest that in the context of AD where the adaptive arms of the immune system weaken, such stimulation of HSP60-specific T cells would not put patients at risk of autoimmune diseases but may rather provide beneficial stimulatory effects for the aging immune system. This is, of course, provided that the proper adjuvant and doses are used.

Elan pharmaceuticals used the QS21 adjuvant in their Aβ1–42 vaccination clinical trial AN-1792 [14]. Considering the increased frequency of Aβ-reactive T cells in some patients with AD compared to adult individuals [19], it is reasonable to speculate that the adjuvant had a key role in their stimulation towards pathogenic T cells, which may have promoted the development of meningoencephalitis observed in about 6% of the vaccinated patients [16]. The HSP60-derived peptide p458 used in our study to provide the T-cell epitope has in itself been shown in previous studies to have adjuvant properties. Notably, vaccines against pneumococcal and meningococcus infection combining the p458 peptide conjugated to bacterial-derived capsular polysaccharides were effective when administrated without an additional adjuvant [34,35]. It was further demonstrated that p458 has an intrinsic adjuvant-like effect via stimulating Toll-like Receptor 4 (TLR4) signaling and inducing prolonged antigen presentation of the antigens on the surface of Antigen Presenting Cells (APCs) [34]. The roles of mammalian HSP60 and HSP70 as endogenous adjuvants have been shown in several studies demonstrating the activation of macrophages and dendritic cells primarily via TLR2 and TLR4 [44,45,48]. It is therefore possible that certain formulations of Aβ–HSP60 will allow its use for AD vaccination without administration of any additional adjuvant, hence further minimizing the risk of pathogenic T-cell activation. Possible carriers and routes of administration need to be further studied in animal models of AD.

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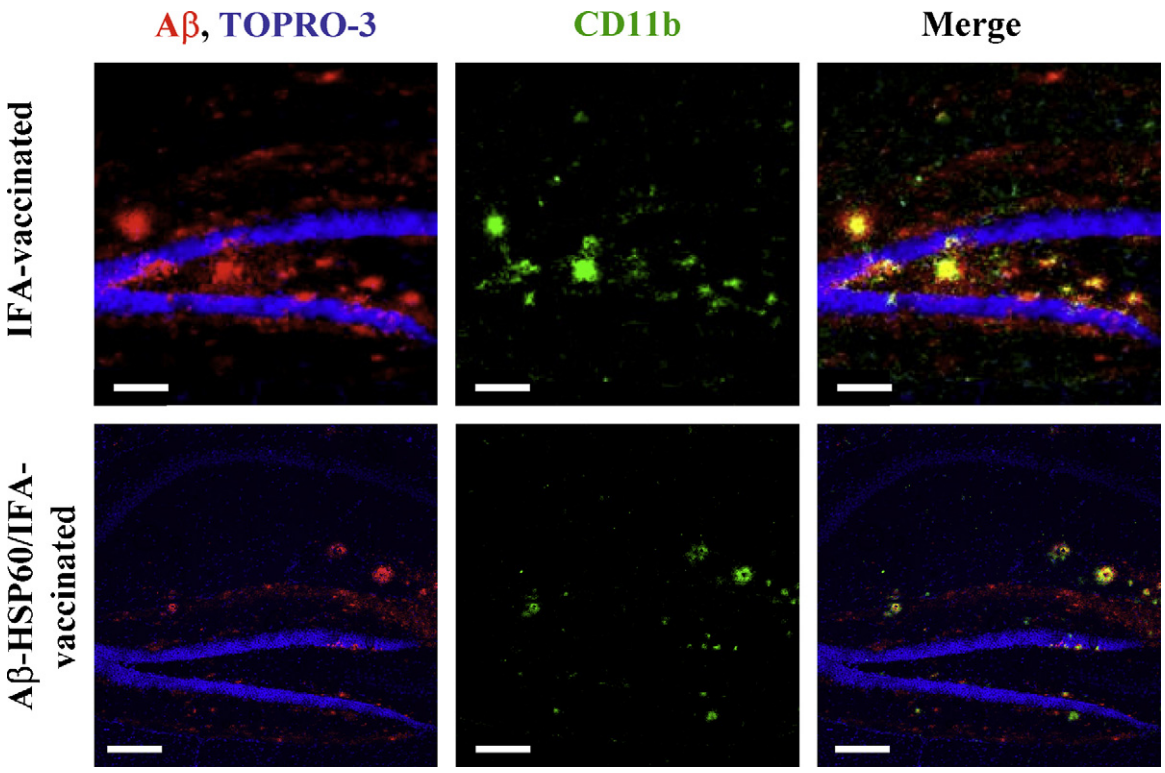

Fig. 6. Decreased microglial activation associated with Aβ plaque clearance in the brains of APP/DR15 Tg mice. Brain sections derived from adjuvant- (upper panels) and Aβ- (lower panels) vaccinated mice were immunolabeled with anti-CD11b (green) and anti-Aβ (red) and counterstained with TO-PRO 3 (blue), as described in Section 2. Three-dimensional Z-stack images taken from the hippocampal area of representative sections show separate panels of CD11b (green) and Aβ (red), and their merged appearance. Bars represent the distance of 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Using a peptide such as p458 requires the validation of its immunogenic characteristics in the context of HLA alleles. We have recently shown that Aβ1–42 itself has several immunodominant epitopes in mice carrying different genetic backgrounds [42] and that these are quite different from the Aβ T-cell epitopes determined in humans [19,20]. The epitopes in humans were mostly in the C-terminus and were HLA-DR dependent. Here we examined the immunogenicity of Aβ–HSP60 vaccine in DR15-Tg mice carrying the highly frequent DR1501 allele where Aβ evoked a robust immune response via the immunodominant T-cell epitope Aβ28–42 [20]. Compared with Aβ1–42 immunization, Aβ–HSP60 elicited a substantially milder immune response where both proinflammatory cytokines IFN-γ and IL-17A were substantially lower. Furthermore, long-term immunization of APP/DR15 Tg mice with Aβ–HSP60 showed that despite the mild HSP60-specific T-cell response, Aβ specific antibody titers were gradually increased and sufficient to promote the clearance of Aβ.

The recent clinical results of treating AD patients by either passive or active immunotherapy demonstrated that this approach is far more complicated than originally thought. However, at present it is still one of the promising approaches for the treatment of this devastating disease. Inducing an immune response against Aβ remains a fundamental strategy for elimination of one of the key players in the disease pathology – the amyloid fibrils. Nonetheless, one should note that the beneficial effects shown by the clearance of Aβ in mouse models of the disease may be partially misleading as they do not necessarily represent the neuronal/synapse loss of neurons possibly preceding the accumulation of Aβ in AD patients. Aβ clearance should therefore be considered as only part of the therapy. Stimulating an immune response that promotes Aβ clearance as well as neuronal repair such as via cytokines and neurotrophic factors [49–53] may be considered as a more appropriate goal of AD immunotherapy.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.vaccine.2011.03.033.

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