Low Concentrations of Anti-Aβ Antibodies Generated in Tg2576 Mice by DNA Epitope Vaccine Fused with 3C3d Molecular Adjuvant Do Not Affect AD Pathology

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Abstract

It has been demonstrated that an active vaccination strategy with protein- or DNA-based epitope vaccines composed of the immunodominant self B cell epitope of amyloid-β_{42} (Aβ_{42}) and a non-self T helper (Th) cell epitope is an immunotherapeutic approach to preventing or treating Alzheimer's disease (AD). As a DNA-based epitope vaccine, we used a plasmid encoding three copies of Aβ_{1-11} and Th cell epitope, PADRE (p3Aβ_{1-11}-PADRE). We have previously reported that three copies of component of complement C3d (3C3d) acts as a molecular adjuvant significantly enhancing immune responses in wild-type mice of the H2^b haplotype immunized with p3Aβ_{1-11}-PADRE. Here, we tested the efficacy of p3Aβ_{1-11}-PADRE and the same vaccine fused with 3C3d (p3Aβ_{1-11}-PADRE-3C3d) in a transgenic (Tg) mouse model of AD (Tg2576) of the H2^{bxs} immune haplotype. The overall responses to both vaccines were very weak in Tg2576 mice despite the fact that the 3C3d molecular adjuvant significantly enhanced the anti-Aβ response to 3Aβ_{1-11}-PADRE. Importantly, generation of low antibody responses was associated with the strain of amyloid precursor protein Tg mice rather than with a molecular adjuvant, as a p3Aβ_{1-11}-PADRE-3C3d vaccine induced significantly higher antibody production in another AD mouse model, 3xTg-AD of the H2^b haplotype. Finally, this study demonstrated that low concentrations of antibodies generated by both DNA vaccines were not sufficient for the reduction of Aβ pathology in the brains of vaccinated Tg2576 animals, confirming previous reports from preclinical studies and the AN-1792 clinical trials, which concluded that the concentration of anti-Aβ antibodies may be essential for the reduction of AD pathology.

Introduction

PLASMID DNA REPRESENTS an attractive platform for the development of vaccines against a variety of pathogens as well as for human diseases, including cancer, autoimmune disorders, and Alzheimer's disease (AD). Several characteristics make naked DNA a desirable route for vaccination: simplicity in manipulation, induction of antigen-specific T and B cell responses similar to those elicited by live attenuated platforms, ease of rapid large-scale production and formulation, long shelf life, and the fact that they are more temperature-stable than conventional vaccines. Although DNA vaccines have generally been very effective mostly in preclinical studies, current results imply that they could also be promising in large animals (Fynan *et al.*, 1993; Robinson *et al.*, 1993; Donnelly and Ulmer, 1999; Powell, 2004) and even in humans (MacGregor *et al.*, 1998; Boyer *et al.*, 1999, 2000; Conry *et al.*, 2002; Epstein *et al.*, 2004; Miller *et al.*, 2005) if a right delivery system and molecular adjuvants are used (Babiuk *et al.*, 2002; Otten *et al.*, 2004; Scheerlinck *et al.*, 2004; Luxembourg *et al.*, 2007). In fact, promising results have been reported in Phase I and Phase II trials for malaria, tuberculosis, and HIV, the three main diseases that have defied the development of effective vaccines to date (MacGregor *et al.*, 1998; Le *et al.*, 2000; Rottinghaus *et al.*, 2003).

Having this data in mind, several years ago, we suggested DNA vaccination as a strategy for generating an AD vaccine (Ghochikyan *et al.*, 2003; Cribbs and Agadjanyan, 2005). AD is a devastating disease that currently affects about 5.3 million people in the United States (http://www.alz.org), and

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is the most common form of dementia, exhibiting progressive memory loss and general cognitive decline. Current data suggest that the accumulation of neurotoxic amyloid-β (Aβ) may play a central role in the onset and progression of AD (Hardy and Higgins, 1992). Accordingly, many therapies for AD are aimed at reducing the level of Aβ in the brain and/or blocking the assembly of Aβ peptide into pathological forms that disrupt cognitive function (Haass and Selkoe, 2007). Thus, a potentially powerful strategy is immunotherapy with anti-Aβ antibody because it can facilitate the reduction of pathological forms of Aβ in the brains of mice (Schenk et al., 1999; Bard et al., 2000; Janus et al., 2000; Morgan et al., 2000; Dodart et al., 2002) and humans (Nicoll et al., 2003, 2006; Ferrer et al., 2004; Masliah et al., 2005a; Patton et al., 2006; Boche et al., 2007; Nitsch and Hock, 2007; Holmes et al., 2008).

However, the first immunotherapy clinical trial was halted because a subset of individuals immunized with the Aβ_{42} peptide formulated in T helper (Th) 1-type adjuvant developed aseptic meningoencephalitis (Hock et al., 2003; Nicoll et al., 2003, 2006; Orgogozo et al., 2003; Ferrer et al., 2004; Bayer et al., 2005; Masliah et al., 2005b; Patton et al., 2006; Holmes et al., 2008). It was suggested that these adverse events in the central nervous system were associated with autoreactive T cells specific to the T cell epitope in Aβ, the conventional adjuvant, and/or the reformulation of the vaccine with polysorbate 80 (Schenk, 2002; Nicoll et al., 2003; Ferrer et al., 2004; Holmes et al., 2008). In addition, there was only a low percentage of elderly participants who responded to the AN-1792 vaccine by producing titers $\geq$1:2,200 of anti-Aβ antibodies (Gilman et al., 2005; Patton et al., 2006). To avoid problems associated with active immunization of elderly AD patients, we previously suggested an active vaccination strategy using peptide- or DNA-based epitope vaccines (Agadjanyan et al., 2005; Cribbs and Agadjanyan, 2005; Mamikonyan et al., 2007; Petrushina et al., 2007; Movsesyan et al., 2008a,b). More specifically, we proposed a DNA epitope vaccine expressing three copies of the immunodominant self B cell epitope (Aβ_{1-11}) and a non-self Th cell epitope, PADRE (p3Aβ_{1-11}-PADRE) instead of the whole Aβ_{42} antigen (Ghochikyan et al., 2003). Of note, to augment the humoral immune response, we fused this DNA epitope vaccine with molecular adjuvants: either the macrophage-derived chemokine (MDC) or three copies of C3d component of complement (3C3d). Although we have already tested the efficacy of plasmid MDC-3Aβ_{1-11}-PADRE in the 3xTg-AD mouse model of AD, the reduction of AD-like pathology has not been previously assessed in transgenic mice immunized with plasmid encoding 3Aβ_{1-11}-PADRE-3C3d. Thus, in this study we tested the immunological and therapeutic efficacy of a DNA epitope vaccine encoding 3Aβ_{1-11}-PADRE-3C3d in a Tg2576 mouse model of AD (Hsiao et al., 1996). We demonstrated that this vaccine was capable of eliciting only low titers of Aβ-specific antibodies in Tg2576 mice. More importantly, this study demonstrated that low concentrations of antibody are not sufficient for the reduction of AD-like pathology in this mouse model of AD. Of note, the low titers of antibodies were associated with the Tg2576 AD mouse model, rather than with the molecular adjuvant, as 3Aβ_{1-11}-PADRE-3C3d induced strong anti-Aβ antibody responses in another AD mouse model (3xTg-AD).

Materials and Methods

Mice

Three- to four-month-old Tg2576 (B6/SJL F1 background, H2^{bxs} haplotype) and 3–4-month-old 3xTg-AD (H2^{b} haplotype) mice were bred and provided by the facility associated with the Alzheimer's Disease Research Center (ADRC) at the University of California, Irvine (UCI). Animals were housed at a temperature- and light-cycle- controlled animal facility at the Institute for Memory Impairments and Neurological Disorders, UCI. All procedures were performed according to the UCI Institutional Animal Care and Use Committee (IACUC) approved protocols and in accordance with guidelines of the National Institutes of Health for the proper care of laboratory animals.

Generation of plasmids

The generation of plasmids tested in the current study has been previously described (Movsesyan et al., 2008b). Plasmids encoding 3Aβ_{1-11}-PADRE-3C3d, 3Aβ_{1-11}-PADRE, and MDC-3Aβ_{1-11}-PADRE were purified by using a Qiagen plasmid maxi kit (Qiagen, Valencia, CA). The purity and concentration of DNA were measured by DNA gel electrophoresis and optical density readings at 260/280 nm.

Immunizations of animals and detection of antibodies

Three- to four-month-old female Tg2576 mice were divided into two treatment groups: p3Aβ_{1-11}-PADRE-3C3d ($n = 9$) and p3Aβ_{1-11}-PADRE ($n = 9$). An additional group of naïve (nonimmunized, $n = 9$) Tg2576 mice of the same gender was kept in the same conditions throughout the entire experiment. Experimental groups received two initial biweekly immunizations and followed up with seven consecutive immunizations at 1.5-month intervals (total of nine injections) using gene gun. Mice were terminated at the age of 17–18 months 10 days after the last immunization. Gene gun immunizations (10 μg per mouse per immunization) were performed exactly as we had reported in prior studies (Ghochikyan et al., 2003; Movsesyan et al., 2008a,b). Anti-Aβ antibody concentrations and antibody isotypes were detected by ELISA exactly as previously described (Cribbs et al., 2003; Ghochikyan et al., 2003; Movsesyan et al., 2008a).

We also performed additional gene gun immunizations of 3–4-month-old Tg2576 and 3xTg-AD female mice with pMDC-3Aβ_{1-11}-PADRE and p3Aβ_{1-11}-PADRE-3C3d (10 μg per mouse for both vaccines, $n = 6$ per each group). Mice were immunized three times with biweekly intervals. Sera were collected on day 10 after the last immunization, and anti-Aβ antibody response was measured by ELISA.

Biochemical and immunohistochemical analysis of mouse brains

Brains of mice were collected for neuropathological analysis exactly as previously described (Petrushina et al., 2007). In brief, Tg2576 mice from all three groups were anesthetized with Nembutal 150 mg/kg and transcardially perfused with ice-cold PBS. Each mouse's skullcap was surgically removed; following this the brain was removed and bisected sagittally. The left hemisphere was snap-frozen and reserved for biochemical analysis, whereas the right hemisphere was fixed

in phosphate-buffered 4% paraformaldehyde at $+4°C$ for 36 hr for further immunohistochemical analysis.

The quantification of the levels of both soluble and insoluble (biochemical analysis) $A\beta_{40}$ and $A\beta_{42}$ in the brain was performed as described previously (Petrushina et al., 2007; Movsesyan et al., 2008a,b). In brief, after the extraction of the soluble fraction, protein concentration was determined using the BCA Protein Assay Kit (Pierce, Rockford, IL), and samples were adjusted to an equal concentration and analyzed using Biosource ELISA kits (Invitrogen, Carlsbad, CA). The insoluble fractions were neutralized and analyzed using Biosource ELISA kits (Invitrogen).

Immersion-fixed hemibrains were coronally sectioned at $50\,\mu m$ and used for immunohistochemical analyses exactly as previously described (Petrushina et al., 2007; Movsesyan et al., 2008a). To detect $A\beta$ deposits, the 6E10 monoclonal antibody (1:1,000; Signet, Dedham, MA) was used on sections pretreated earlier with 90% formic acid for 4 min. To quantify the number of $A\beta$ plaques, six images of the cortical area and six images of hippocampal areas per mouse were taken using a 20× objective with a Sony high-resolution CCD video camera (XC-77) and manually counted. In addition, NIH image analysis was performed using NIH 1.59b5 software. The threshold intensity was manually set and kept constant. Quantification was performed by a single examiner blind to the identity of the samples.

Statistical analysis

All statistical parameters (mean, standard deviation, significant difference) were calculated using Prism 3.03 software (GraphPad Software, Inc., San Diego, CA). Statistically significant differences were examined using a t test or analysis of variance (one-way ANOVA) with Tukey's multiple comparisons posttest ($p < 0.05$ was considered significantly different).

Results and Discussion

To investigate the potency of the 3C3d molecular adjuvant, we immunized 3–4-month-old Tg2576 mice with $p3A\beta_{1-11}$-PADRE-3C3d or $p3A\beta_{1-11}$-PADRE. It has been shown that as Tg2576 animals age, $A\beta$ is altered beginning at the age of 6–7 months (Hsiao et al., 1996; Kawarabayashi et al., 2001). Therefore, immunization started at a young age is intended to prevent $A\beta$ deposition in the brains and slow disease progression by the age of 17–18 months. The first two immunizations (initiated at the age of 3–4 months) were performed biweekly followed by booster injections at 1.5-month intervals (a total of nine). Mice were killed at the age of 17–18 months 10 days after the last immunization. Blood was collected following each immunization, and the humoral immune response was evaluated in sera. DNA vaccine, $p3A\beta_{1-11}$-PADRE, induced a detectable antibody response to $A\beta_{42}$ in all of the mice after the first immunization ($333.24 \pm 84.2\,ng/ml$; Fig. 1). The fusion of this vaccine with the 3C3d molecular adjuvant significantly enhanced the humoral immune response, reaching $711.86 \pm 125.7\,ng/ml$ (Fig. 1). After the booster injection with the epitope vaccine candidates, $p3A\beta_{1-11}$-PADRE and $p3A\beta_{1-11}$-PADRE-3C3d, Tg2576 mice generated $273.15 \pm 54.7\,ng/ml$ and $571.76 \pm 78.9\,ng/ml$ of anti-$A\beta$ antibodies, respectively (Fig. 1). Subsequent booster injections of the experimental animals did
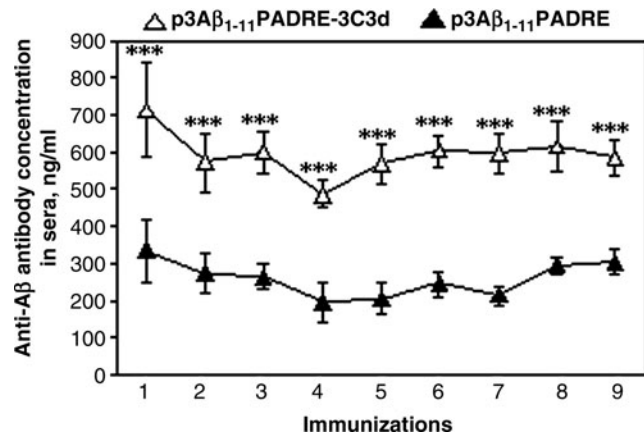

FIG. 1. $3A\beta_{1-11}$-PADRE epitope vaccine with and without 3C3d molecular adjuvant induced low titers of anti-$A\beta$ antibodies in Tg2576 mice. Preventive immunizations of Tg2576 mice (initiated at 3–4 months of age and carried up to 17–18 months) with $p3A\beta_{1-11}$-PADRE induced poor production of anti-$A\beta$ antibodies. Addition of 3C3d molecular adjuvant to $p3A\beta_{1-11}$-PADRE plasmid ($p3A\beta_{1-11}$-PADRE-3C3d) significantly enhanced humoral immune response (***$p < 0.001$); however, the strength of the humoral immune response in both immunized groups was low.

not increase the concentrations of anti-$A\beta$ antibody and maintained the low levels of antibodies generated after the second immunization with $p3A\beta_{1-11}$-PADRE-3C3d and $p3A\beta_{1-11}$-PADRE. However, the difference in the strength of generated humoral immune responses in both groups after all injections was statistically significant (Fig. 1).

Next, to analyze the isotype profile of the humoral immune response, we detected the subclass of antibodies (IgG1, IgG2a[b], IgG2b, IgM). Three immunizations of Tg2576 mice with $p3A\beta_{1-11}$-PADRE-3C3d induced an increased production of IgM, a substantial amount of IgG1, and low levels of both IgG2a[b] and IgG2b (Fig. 2A). Meanwhile, the isotype profile in the group of mice immunized with $p3A\beta_{1-11}$-PADRE looked different. There was a substantial amount of both IgG1 and IgG2a[b] isotypes and high production of the IgM isotype. In addition, we detected a considerably high level of the IgG2b isotype (Fig. 2A). We measured the IgG1/IgG2a[b] ratio, which is an indirect indicator of a Th1- or Th2-type immune response (Snapper and Paul, 1987; Finkelman et al., 1990; Hasbold et al., 1999). The IgG1/IgG2a[b] ratio in both groups of Tg2576 mice immunized with $p3A\beta_{1-11}$-PADRE-3C3d (2.02 ± 0.3) or $p3A\beta_{1-11}$-PADRE (1.02 ± 0.15) was greater than 1 (Fig. 2B). Thus, we did not observe significant differences in the type of humoral response generated after vaccination with $p3A\beta_{1-11}$-PADRE-3C3d versus $p3A\beta_{1-11}$-PADRE.

To study the effect of the tested DNA vaccines on neuropathology, two groups of immunized animals and non-immunized control mice were killed at the age of 17–18 months and their brain sections were used for immunohistochemical analysis. Imaging analysis showed the same level of immunoreactivity to 6E10 in both experimental groups as well as in the nonimmunized control group (Fig. 3). Even though immunization with the epitope vaccine with or without the molecular adjuvant was not effective in reducing
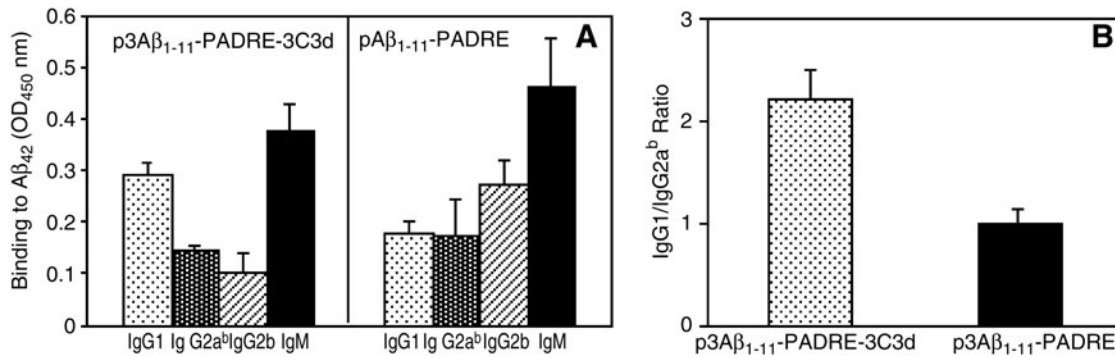
FIG. 2. Isotype profile of Aβ-specific antibodies generated in transgenic Tg2576 mice. **(A)** Immunizations of Tg2576 mice with DNA epitope vaccines (p3Aβ_{1-11}-PADRE-3C3d or p3Aβ_{1-11}-PADRE) induce production of IgG1, IgG2a^b, and IgG2b isotypes. Interestingly, a high production of IgM isotypes was detected in both groups of experimental mice. **(B)** Calculation of the IgG1/IgG2a^b ratio determined a slight Th2-biased humoral immune response in the group immunized with p3Aβ_{1-11}-PADRE, whereas addition of 3C3d molecular adjuvant induced a stronger Th2-type of immune response (group p3Aβ_{1-11}-PADRE-3C3d).

the amyloid burden in the brains of immunized animals, we still decided to determine whether there were changes in the levels of both soluble and insoluble A$\beta_{40/42}$. The results revealed no statistically significant changes in the SDS-soluble and SDS-insoluble (formic acid) extracts for both Aβ_{40} and Aβ_{42} between the experimental and the control groups (Fig. 4). These findings indicate that the titer of antibodies generated in Tg2576 mice in response to immunization with DNA epitope vaccines (p3Aβ_{1-11}-PADRE-3C3d and p3Aβ_{1-11}-PADRE) was not sufficient to affect the amyloid clearance in the brains of these mice.
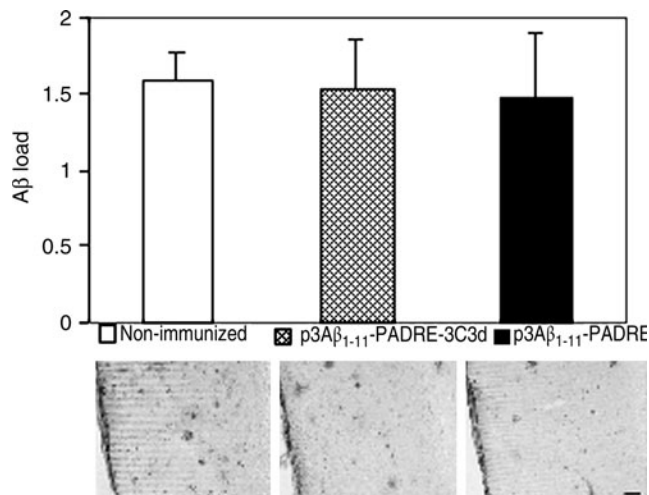

FIG. 3. Immunizations with either vaccine candidate did not change Aβ plaque load in the brains of Tg2576 mice. Analysis of Aβ plaque load was performed on brain sections of transgenic mice terminated at 17–18 months of age. Preventive immunizations did not affect plaque deposition in the brains of mice from both vaccinated groups. Representative staining images of the distribution of Aβ plaques stained with 6E10 monoclonal antibody in the cortex of nonimmunized and p3Aβ_{1-11}-PADRE-3C3d- and p3Aβ_{1-11}-PADRE-immunized mice are presented. Scale bar: 200 μm at 4× magnification.

Previously, we reported positive correlation between the concentration of anti-Aβ antibody and the reduction of insoluble, cerebral Aβ plaques in Tg2576 mice immunized with a peptide-based epitope vaccine (Petrushina *et al.*, 2007). Our current data with Tg2576 mice vaccinated with p3Aβ_{1-11}-PADRE-3C3d and p3Aβ_{1-11}-PADRE support these results. It is worth mentioning that the data from clinical trials also reported a positive correlation between levels of anti-Aβ antibodies and the reduction of Aβ pathology (Nicoll *et al.*, 2003; Ferrer *et al.*, 2004; Bayer *et al.*, 2005; Gilman *et al.*, 2005). Future clinical trials with active and passive vaccination may shed more light on the connection between antibody titers, pathology, and the neurological outcome.

Thus, neither p3Aβ_{1-11}-PADRE nor p3Aβ_{1-11}-PADRE-3C3d induced a strong anti-Aβ antibody response in Tg2576 mice. Although the 3C3d molecular adjuvant fused with a DNA epitope vaccine enhanced humoral immune response significantly (Fig. 1), it still induced very low responses in these mice. It is well known that a major function of C′ is the opsonization of antigens/immune complexes. This is mediated by the covalent attachment of activated C3d and C3dg fragments of C′ to the antigen, which links the innate and the adaptive immune responses by targeting the immunogen to specific C′ receptors type 1 (CD35) and type 2 (CD 21) (Carroll, 1998; Fearon and Carroll, 2000). CD21 is expressed on mature B cells as a noncovalent complex with CD19 and functions as a specialized membrane adaptor protein for antigen-specific B cell receptors (BCR) (Ahearn *et al.*, 1996; Croix *et al.*, 1996). The simultaneous engagement of BCR and CD21/CD19 by the antigen-C3d complex dramatically reduces the BCR:antigen affinity threshold, significantly prolongs BCR residency in lipid rafts, and induces much stronger B cell activation (Carter and Fearon, 1992; Mongini *et al.*, 1997; Cherukuri *et al.*, 2001). The immunization of mice with recombinant hen egg lysozyme fused with 3C3d dramatically enhanced antibody formation (Dempsey *et al.*, 1996). DNA vaccines encoding various antigens fused with 3C3d also increased antibody responses (Ross *et al.*, 2000, 2001; Mitchell *et al.*, 2003; Tong *et al.*, 2006). We also reported that p3Aβ_{1-11}-PADRE induced strong anti-Aβ antibody response in C57Bl/6 mice, and that 3C3d significantly en-
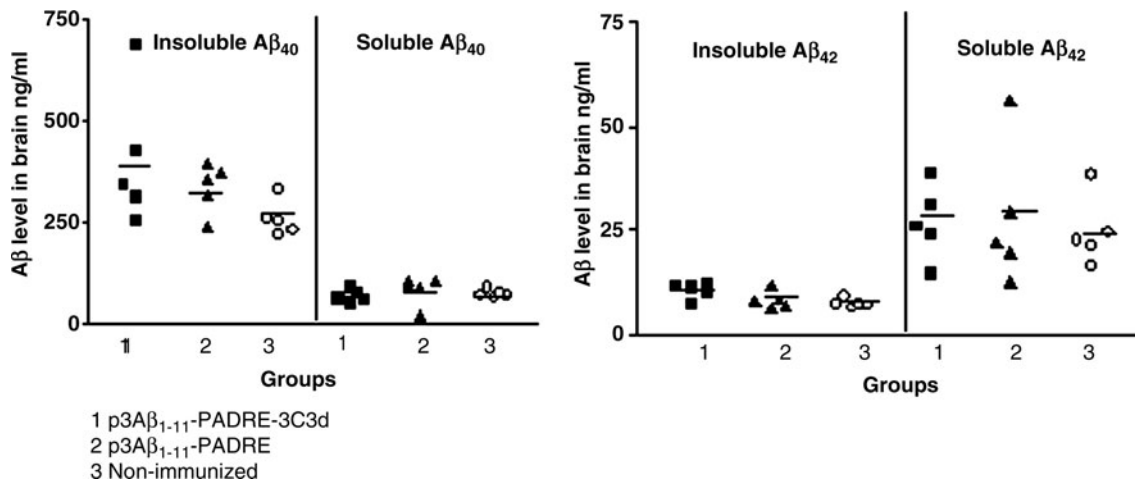
FIG. 4. Biochemical analysis of brain tissues revealed no changes in soluble and insoluble $A\beta$ levels in both immunized and nonimmunized groups of Tg2576 mice. Low titers of anti-$A\beta$ antibodies generated after immunization with p3Aβ_{1-11}-PADRE-3C3d or p3Aβ_{1-11}-PADRE were not sufficient for inhibition of both soluble and insoluble $A\beta_{40}$ and $A\beta_{42}$ peptide levels in the brain. Analysis of neuropathological changes was performed in the brains of vaccinated and control (nonimmunized) mice at the age of 17–18 months.

hanced this response (Movsesyan *et al.*, 2008b). However, here we demonstrated that although the fusion of p3Aβ_{1-11}-PADRE with 3C3d enhanced the humoral immune response, both vaccines (p3Aβ_{1-11}-PADRE and p3Aβ_{1-11}-PADRE-3C3d) induced only weak immune responses in the Tg2576 mouse model of AD. One idea is that this low response could be associated with differences in the immune haplotype of C57Bl/6 (H2^b) versus Tg2576 (H2^{bxs}) mice or with some other immune functions linked to the transgenic characteristics of the mice. The hyporesponsiveness of Tg2576 mice to $A\beta_{42}$ due to the induction of tolerance to self T cell epitope was demonstrated earlier by different groups including ours (Monsonego *et al.*, 2001; Petrushina *et al.*, 2003). Here we demonstrated for the first time that Tg2576 animals also do not respond to immunizations with a vaccine composed of foreign T cell epitope and self-B cell epitope, $A\beta_{1-11}$.

Previously we reported that the same p3Aβ_{1-11}-PADRE vaccine fused with another molecular adjuvant, MDC, was effective in generating high concentrations of anti-$A\beta$ antibodies in a wild-type (C57Bl/6) and triple transgenic mouse model of AD (Movsesyan *et al.*, 2008a). Thus, to understand the underlying reason of such low efficacy of p3Aβ_{1-11}-PADRE-3C3d in Tg2576 mice here, we compared immune responses generated in Tg2576 and 3xTg-AD mouse models of AD immunized with the same vaccine, which was fused with either MDC or 3C3d molecular adjuvants. Results presented in Table 1 demonstrated that both pMDC-3Aβ_{1-11}-PADRE and p3Aβ_{1-11}-PADRE-3C3d vaccines induced significantly stronger immune responses in 3xTg-AD mice than in the Tg2576 mouse model of AD ($p < 0.01$ and $p < 0.05$, respectively). It has been reported that T cell epitope, PADRE, is recognized by MHC class II molecules of H2^b mice very well (Alexander *et al.*, 2000). So to exclude the possibility that loss of the H2^b haplotype during breeding could be responsible for the low immune responses in Tg2576 mice of the H2^{bxs} immune haplotype, we immunized both mouse models of AD, which have different genetic backgrounds, with the peptide-based epitope vaccine,

2Aβ_{1-11}-PADRE, synthesized as a multiple antigenic peptide (2Aβ_{1-11}-PADRE-MAP). 3xTg-AD mice of the H2^b haplotype responded to this vaccine very well. More importantly, immunizations with the same peptide-based epitope vaccine induced strong antibody production in Tg2576 mice as well (Table 1). Although this response is slightly lower than in 3xTg-AD mice, this difference was not statistically significant. Of note, SJL mice of the H2^s haplotype practically did not respond to p3Aβ_{1-11}-PADRE fused with 3C3d (data not shown). These results argue that our colony of Tg2576 mice did not lose the H2^b haplotype during breeding. Further analyses are needed to understand why the immunogenicity of the DNA-based, but not of the peptide-based, epitope vaccine is impaired in Tg2576 mice. One of the mechanisms possibly responsible for this outcome may be the induction of B cell tolerance due to a high level of $A\beta$ in the serum of Tg2576 mice, which could be broken by multiple B cell epitopes in the lysine branch of the MAP peptide.

In sum, this study revealed a low potency of the DNA epitope vaccine in Tg2576 mice and confirmed our previously reported data that low concentrations of anti-$A\beta$ antibodies are not sufficient for the reduction of AD-like pathology.

TABLE 1. CONCENTRATION OF ANTI-$A\beta$ ANTIBODY
IN AD MOUSE MODELS IMMUNIZED WITH DNA
AND PEPTIDE BASED EPITOPE VACCINES

	Anti-$A\beta$ antibody concentration ($\mu g/ml$)	
Immunogen	*Tg2576 (H2^{bxs})*	*3xTg-AD (H2^b)*
pMDC-3Aβ_{1-11}-PADRE	1.42 ± 1.45	11.95 ± 5.49
p3Aβ_{1-11}-PADRE-3C3d	2.43 ± 1.68	21.14 ± 17.79
2Aβ_{1-11}-PADRE-MAP peptide/Quil A	141.95 ± 151.3	223.8 ± 65.1

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Author Disclosure Statement

All authors have nothing to disclose.

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