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Prototype Alzheimer’s Disease Vaccine Using the Immunodominant B Cell Epitope from β-Amyloid and Promiscuous T Cell Epitope Pan HLA DR-Binding Peptide

Michael G. Agadjanyan,2* Anahit Ghochikyan,* Irina Petrushina,† Vitaly Vasilevko,† Nina Movsesyan,* Mikayel Mkrtchyan,* Tommy Saing,† and David H. Cribbs2,3†‡

Immunization of amyloid precursor protein transgenic mice with fibrillar β-amyloid (Aβ) prevents Alzheimer’s disease (AD)-like neuropathology. The first immunotherapy clinical trial used fibrillar Aβ, containing the B and T cell self epitopes of Aβ, as the immunogen formulated with QS21 as the adjuvant in the vaccine. Unfortunately, the clinical trial was halted during the phase II stage when 6% of the participants developed meningoencephalitis. The cause of the meningoencephalitis in the patients that received the vaccine has not been definitively determined; however, analysis of two case reports from the AN-1792 vaccine trial suggest that the meningoencephalitis may have been caused by a T cell-mediated autoimmune response, whereas production of anti-Aβ Abs may have been therapeutic to the AD patients. Therefore, to reduce the risk of an adverse T cell-mediated immune response to Aβ immunotherapy we have designed a prototype epitope vaccine that contains the immunodominant B cell epitope of Aβ in tandem with the synthetic universal Th cell pan HLA DR epitope, pan HLA DR-binding peptide (PADRE). Importantly, the PADRE-Aβ1–15 sequence lacks the T cell epitope of Aβ. Immunization of BALB/c mice with the PADRE-Aβ1–15 epitope vaccine produced high titers of anti-Aβ Abs. Splenocytes from immunized mice showed robust T cell stimulation in response to peptides containing PADRE. However, splenocytes from immunized mice were not reactivated by the Aβ peptide. New preclinical trials in amyloid precursor protein transgenic mouse models may help to develop novel immunogen-adjunct configurations with the potential to avoid the adverse events that occurred in the first clinical trial. The Journal of Immunology, 2005, 174: 1580–1586.

A lzheimer’s disease (AD) is the most common form of dementia in the elderly and is characterized by a progressive loss of memory and a general cognitive decline. The neuropathological features of the disease include neurofibrillary tangles, deposition of β-amyloid (Aβ) in senile plaques, and neuronal loss in affected brain regions (1). The Aβ peptide is cleaved from the amyloid precursor protein (APP) by β- and γ-secretases (2–4) and is believed to play an important role in the onset and progression of AD (5, 6). Many strategies currently being proposed as therapies for AD are aimed at reducing the level of Aβ in the brain or blocking the assembly of the peptide into pathological forms (7).

One potentially powerful strategy for reducing the level of Aβ in the brain is immunotherapy, where Aβ-specific Abs facilitate the clearance of Aβ. Active immunization of APP transgenic mice (APP/Tg) with fibrillar Aβ peptide blocked the deposition of Aβ in plaques, prevented the development of dystrophic neurites, and reduced astrogliosis in the mouse brain (8, 9). In addition, when older mice with established Aβ deposits were immunized with Aβ, they were able to clear Aβ plaques from the brain. Other researchers have reported that active immunization protected mice from developing functional memory deficits (10–12), and that passive administration of anti-Aβ mAbs to APP/Tg mice also reduced Aβ levels in the brain (13, 14) and reversed memory deficits (15). These results suggest that the generation of Abs against Aβ in humans might provide similar benefits to patients with AD. There were no reported adverse inflammatory events after Aβ immunotherapy in several animal models, including rabbits, guinea pigs, and monkeys. The only documented adverse incidence to Aβ immunotherapy was an increase in cerebral hemorrhages in very old, hemorrhage-prone, APP/Tg-23 mice injected with multiple high doses of anti-Aβ mAb (16).

Based on the impressive preclinical results, Elan Corp. in collaboration with Wyeth-Ayerst began the first Aβ immunotherapy clinical trial with their AN-1792 vaccine, a mixture of fibrillar Aβ42 and the saponin adjuvant QS-21, on AD patients. Unfortunately, the phase IIa trial was halted when ~6% of the participants in the clinical trial developed aseptic meningoencephalitis (9, 17–20). The cause of the meningoencephalitis in a subset of the patients has not been definitively determined. However, postmortem examination of brains from two patients who suffered an adverse event to the vaccine revealed the presence of T lymphocyte infiltration in the leptomeninges, cerebrovasculature, and cerebral neocortex (21, 22). This has provided support for the theory that the adverse response to the vaccine was due to a T cell-mediated autoimmune response (9, 21–23).
The development of a safe and effective AD vaccine will require a delicate balance between providing specific and adequate anti-\( \alpha \beta \) Ab responses sufficient for therapeutic benefit while eliminating adverse T cell-mediated autoimmune responses. To reduce the risk of an adverse T cell-mediated immune response to \( \alpha \beta \) immunotherapy we have designed a prototype vaccine that will target the immunogenic B cell epitope of \( \alpha \beta \) that is critical for clearance of \( \alpha \beta \) plaques, but that will not stimulate anti-\( \alpha \beta \) T cells. We have engineered an epitope vaccine composed of the B cell epitope from the immunodominant region of \( \alpha \beta_{12}, \alpha \beta_{15} \), in tandem with a universal synthetic T cell epitope, pan HLA DR-binding peptide (PADRE), which consists of a pan HLA DR-binding epitope. We have demonstrated that immunization of BALB/c mice with this epitope vaccine (PADRE-\( \alpha \beta_{12}, \alpha \beta_{15} \)) synthesized as a multiple antigenic peptide (MAP) and formulated in a Th2 type adjuvant (Alum), generated anti-PADRE Th cells (CD4\(^+\)T1/ST2\(^+\)) that support the development of potentially therapeutic anti-\( \alpha \beta \) Abs.

Materials and Methods

Mice

Eight- to 10-wk-old female BALB/c mice were purchased from The Jackson Laboratory. All animals were housed in a temperature- and light cycle-controlled facility, and their care was under the guidelines of the National Institutes of Health and an approved institutional animal care and use committee protocol at University of California (Irvine, CA).

Epitope vaccine, peptide immunogens, and immunizations

To prepare a prototype epitope AD vaccine, we synthesized the N terminus of the immunodominant B cell epitope of \( \alpha \beta_{12}, \alpha \beta_{15} \) (24–35) in tandem with a promiscuous foreign T cell epitope, PADRE. PADRE (aK-Cha-VAAW TLKAla, where a is D alanine, and Cha is L-cyclohexylalanine) is a small 13-mer nonnatural pan HLA DR-binding sequence that is a potent T cell epitope vaccine (PADRE-A\(_{13-mer}\)). We have demonstrated that immunization of BALB/c mice with this universal synthetic T cell epitope, pan HLA DR-binding peptide (PADRE), which consists of a pan HLA DR-binding epitope. We have demonstrated that immunization of BALB/c mice with this epitope vaccine (PADRE-\( \alpha \beta_{12}, \alpha \beta_{15} \)) synthesized as a multiple antigenic peptide (MAP) and formulated in a Th2 type adjuvant (Alum), generated anti-PADRE Th cells (CD4\(^+\)T1/ST2\(^+\)) that support the development of potentially therapeutic anti-\( \alpha \beta \) Abs.

Sera from immunized mice were screened for the ability to bind to \( \alpha \beta \) plaques in the human brain as previously described (32). However, to decrease nonspecific activation of splenocytes, we used HL-1 serum-free synthetic medium (Cambrex) without FBS. Splenocytes from experimental and control mice (5 \( \times \) 10\(^5\) in 100 \( \mu \)l) were restimulated in vitro with different peptides at a concentration of 10 \( \mu \)g/ml. Cells were first incubated for 72 h, then 1 \( \mu \)Ci of \([\text{H}]\)thymidine (Amersham Biosciences) was added to each well for 16–18 h. Cells were harvested using a Tomtec Mach III harvester, and \([\text{H}]\)thymidine uptake (cpm) was counted on a MicroBeta 1450 Trilux scintillation counter (Wallac Oy). The stimulation index was calculated as previously described (47).

Production of cytokines by immune splenocytes

The same splenocytes used for T cell proliferation were used for detection of Th1 (IFN-\( \gamma \)) or Th2 (IL-4) lymphokines as well as for TNF-\( \alpha \) using the ELISPOT technique. Experiments were conducted as recommended by the manufacturer (BD Pharmingen) and as previously described (32). The colored spots were counted, and the differences between stimulated and nonstimulated conditions for each experiment were analyzed statistically as described below.

Detection of CD4\(^+\) T cells expressing IL-18R (Th1) or T1/ST2 (Th2) molecules

Spleens from mice immunized with different immunogens or control animals were dephosphorylated of CD8\(^+\) cells using MACS depletion kit (Miltenyi Biotec), and the remaining splenocytes were restimulated in HL-1 medium with the same peptide that was used for in vivo immunization. On day 0 (baseline) and after 7 days of stimulation, CD4\(^+\) T cells were analyzed for the expression of IL-18R or T1/ST2-selective surface markers using a FACScan flow cytometer (BD Biosciences). The following Abs were used: FITC-labeled anti-mouse T1/ST2 (BD Biosciences), anti-mouse IL-18R\(\beta\) followed by staining with PE-labeled goat anti-rat Ab (BD Pharmingen), and PerCP-labeled anti-mouse CD4 mAb (BD Pharmingen). Data were presented as the percentage of CD4\(^+\) T cells expressing the appropriate marker Ag after specific activation with the indicated peptide minus background activation using an irrelevant peptide.

Statistical analysis

The data for Ab production between groups were analyzed by one-way ANOVA. The results of ELISPOT were examined for differences between groups immunized and restimulated with the indicated peptides using one-way ANOVA and Tukey’s pairs comparison post-test (PRISM 3.03; GraphPad).

Results

PADRE-\( \alpha \beta_{12}, \alpha \beta_{15} \) epitope vaccine induces high titers of anti-\( \alpha \beta \) Abs

Differences in the anti-\( \alpha \beta \) Abs titers produced in response to PADRE-\( \alpha \beta_{12}, \alpha \beta_{15} \)-MAP, \( \alpha \beta_{15-MAP}, \alpha \beta_{13-MAP}, \) and \( \alpha \beta_{15-MAP} \) immunogens were analyzed in sera from immunized animals after each boost. Surprisingly, the best immunogen initially was \( \alpha \beta_{13-MAP} \) which contains both the B and T cell epitopes of \( \alpha \beta_{15} \) (Fig. 1). As expected, linear \( \alpha \beta_{15} \) was not immunogenic because it lacks a T cell epitope for mice of the H-2\(^d\) haplotype (32). The profile of Ab production in the group of mice immunized with PADRE-\( \alpha \beta_{15-MAP} \) epitope vaccine was similar to that of mice immunized with linear \( \alpha \beta_{13-MAP} \) peptide (Fig. 1). After the second boost, all mice from these two groups generated significant titers of anti-\( \alpha \beta \) Abs,
Specific only to A/H9252 anti-A IgG1, IgG2a, IgG2b, and IgM were measured in response to immunization with A/H9252/H11006 antigens of vaccine. The data obtained with pooled sera demonstrated that immunization with A/H9252 MAP was 1–33 induced anti-A Ab isotypes produced in response to immunization with A/H9252 MAP backbone (data not shown). Thus, the T cell epitope PADRE (48) promoted the production of Abs against the Aβ1–15 B cell antigenic determinant of A/H9252 MAP. The Aβ1–15-MAP induced significantly higher anti-Aβ Ab production at wk 3, 5, and 7 compared with PADRE-Aβ1–15-MAP and Aβ1–33 (p < 0.001 to p < 0.05). At wk 9, the data are significant only between groups of mice immunized with Aβ1–33-MAP and Aβ1–33 (p < 0.05), whereas at wk 13, no significant differences among these three groups were observed. Mice immunized with Aβ1–15 (n = 8) did not induce anti-Aβ Abs, and sera from all immunized animals did not recognize wells of ELISA plates coated with PADRE (OD, 0.046 ± 0.003).

Ab isotypes produced in response to immunization with Aβ1–23, Aβ1–33, and PADRE-Aβ1–15-MAP

We measured the production of IgG1, IgG2a, IgG2b, and IgM anti-Aβ Abs in BALB/c mice of H-2d haplotype. The arrows indicate the times of immunization/boosting of the mice. Three groups of mice (n = 9/group) immunized with Aβ1–33-MAP, PADRE-Aβ1–15-MAP, and Aβ1–33 induced anti-Aβ Abs. At wk 13, the range of concentrations was 154–311 μg/ml (average titer, 1/32,000 for PADRE-Aβ1–15-MAP or Aβ1–33-MAP, and 1/64,000 for Aβ1–33-MAP). The Aβ1–33-MAP induces significantly higher anti-Aβ Ab production at wk 3, 5, and 7 compared with PADRE-Aβ1–15-MAP and Aβ1–33 (p < 0.001 to p < 0.05). At wk 9, the data are significant only between groups of mice immunized with Aβ1–33-MAP and Aβ1–33 (p < 0.05), whereas at wk 13, no significant differences among these three groups were observed. Mice immunized with Aβ1–15 (n = 8) did not induce anti-Aβ Abs, and sera from all immunized animals did not recognize wells of ELISA plates coated with PADRE. 

FIGURE 1. Epitope vaccine composed of B cell antigenic determinant (Aβ1–15) and T cell antigenic determinant (PADRE)-induced potent anti-Aβ Abs in BALB/c mice of H2d haplotype. The arrows indicate the times of immunization/boosting of the mice. Three groups of mice (n = 9/group) immunized with Aβ1–33-MAP, PADRE-Aβ1–15-MAP, and Aβ1–33 induced anti-Aβ Abs. At wk 13, the range of concentrations was 154–311 μg/ml (average titer, 1/32,000 for PADRE-Aβ1–15-MAP or Aβ1–33-MAP, and 1/64,000 for Aβ1–33-MAP). The Aβ1–33-MAP induces significantly higher anti-Aβ Ab production at wk 3, 5, and 7 compared with PADRE-Aβ1–15-MAP and Aβ1–33 (p < 0.001 to p < 0.05). At wk 9, the data are significant only between groups of mice immunized with Aβ1–33-MAP and Aβ1–33 (p < 0.05), whereas at wk 13, no significant differences among these three groups were observed. Mice immunized with Aβ1–15 (n = 8) did not induce anti-Aβ Abs, and sera from all immunized animals did not recognize wells of ELISA plates coated with PADRE (OD, 0.046 ± 0.003).

Potential therapeutic efficacy of anti-Aβ Abs generated in response to PADRE-Aβ1–15 epitope vaccine

To demonstrate the potential therapeutic efficacy of anti-Aβ Abs generated in mice immunized with our PADRE-Aβ1–15-MAP epitope vaccine, we analyzed binding of antisera to Aβ plaques in brain tissue from an AD case. The results demonstrate that the antisera from sera collected from individual mice immunized with Aβ1–33-MAP or PADRE-Aβ1–15-MAP bound equally well to Aβ plaques even at a dilution of 1/1000, the end point dilution used in these experiments, which was similar in intensity to the immunostaining with mAb 6E10 (dilution 1/1500). Sera collected from mice immunized with linear Aβ1–15 did not bind to plaques (Fig. 3). These data suggest that anti-Aβ Abs raised in mice immunized with the PADRE-Aβ1–15-MAP
epitope vaccine are potentially therapeutic. In addition, affinity-purified anti-\( \alpha \)\( \beta \) Abs from mice immunized with the epitope vaccine blocked the assembly of \( \alpha \)\( \beta \)\(_{1-15} \) into fibrils in vitro (data not shown).

**PADRE- and \( \alpha \)\( \beta \)-specific T cell responses in splenocytes from immunized mice**

One challenge associated with the clinical use of the \( \alpha \)\( \beta \) self T cell epitope as part of a vaccine to treat AD patients is the potential for the development of unwanted anti-\( \alpha \)\( \beta \) or anti-APP Th1 immune responses (20–22, 32). Thus, an important test of our PADRE-\( \alpha \)\( \beta \)\(_{1-15}-\)MAP epitope vaccine was to demonstrate that T cell responses against PADRE-\( \alpha \)\( \beta \)\(_{1-15}-\)MAP immunoconjugate generated the highest IL-2 response, whereas mice immunized with PADRE-\( \alpha \)\( \beta \)\(_{1-15}-\)MAP had intermediate and low responses (1–33 does not induce anti-\( \alpha \)\( \beta \)-specific T cell proliferation in response to \( \alpha \)\( \beta \)\(_{40}-\)peptide. Interestingly, both \( \alpha \)\( \beta \)\(_{40}-\) and \( \alpha \)\( \beta \)\(_{1-33}-\)MAP Ags were equally potent in anti-\( \alpha \)\( \beta \) T cell activation, whereas \( \alpha \)\( \beta \)\(_{20-31}-\)MAP did not induce T cell proliferation. These data demonstrate that \( \alpha \)\( \beta \)\(_{1-33} \) possesses the T cell epitope of \( \alpha \)\( \beta \). Importantly, splenocytes isolated from mice immunized with PADRE-\( \alpha \)\( \beta \)\(_{1-15}-\)MAP induced equally strong T cell proliferation after restimulation with PADRE, PADRE-\( \alpha \)\( \beta \)\(_{1-15} \), or PADRE-\( \alpha \)\( \beta \)\(_{1-15}-\)MAP, but not with \( \alpha \)\( \beta \)\(_{40} \) or \( \alpha \)\( \beta \)\(_{1-33}-\)MAP (Fig. 4A). These results clearly demonstrate that PADRE, but not \( \alpha \)\( \beta \) or MAP peptides, provides the Th lymphocyte support necessary for a strong anti-\( \alpha \)\( \beta \) Ab response to the PADRE-\( \alpha \)\( \beta \)\(_{1-15}-\)MAP epitope vaccine.

**Peptide-induced lymphokine responses in splenocytes from immunized mice**

We analyzed the production of Th1 (IFN-\( \gamma \)) and Th2 (IL-4) lymphokines and TNF-\( \alpha \), a proinflammatory cytokine, in splenocyte cultures from immunized and control mice. Groups of mice injected with \( \alpha \)\( \beta \)\(_{1-33}-\)MAP induced the highest IL-4 response, whereas mice immunized with \( \alpha \)\( \beta \)\(_{40} \) and PADRE-\( \alpha \)\( \beta \)\(_{1-15}-\)MAP had intermediate and low responses (\( p < 0.05 \) and \( p < 0.001 \), respectively). PADRE-\( \alpha \)\( \beta \)\(_{1-15}-\)MAP induced the highest IFN-\( \gamma \) response based on the number of cells producing this lymphokine, which was significantly higher than those in the other two groups (\( p < 0.001 \)). Importantly, splenocytes isolated from mice immunized with \( \alpha \)\( \beta \)\(_{40} \) and PADRE-\( \alpha \)\( \beta \)\(_{1-15}-\)MAP did not generate IL4, IFN-\( \gamma \), or TNF-\( \alpha \) cytokines (Fig. 5).

**Expression of Th1 (IL-18R) and Th2 (T1/ST2)-specific markers on CD4\(^+\) T cells from immunized mice**

To further characterize the contributions of Th1 and Th2 CD4\(^+\) T cells to the immune response in immunized BALB/c mice, we analyzed the percentage of CD4\(^+\) T cells expressing either Th1 (IL-18R) or Th2 (T1/ST2)-specific markers. Without in vitro activation, we did not detect a significant number of CD4\(^+\) T cells expressing IL-18R or T1/ST2 molecules. However, restimulation of splenocytes from mice immunized with \( \alpha \)\( \beta \)\(_{1-33}-\)MAP or PADRE-\( \alpha \)\( \beta \)\(_{1-15}-\)MAP generated 18–19% CD4\(^+\) T cells that...
cytes from all groups were depleted by CD8<sup>+</sup> H<sub>11001</sub> vivo injections, except splenocytes from mice immunized restimulated with the same peptides that were used for in

apies for AD are based on reducing the level of A of AD (2–6). Many strategies currently being investigated as ther-

is believed to play an important role in the onset and progression (Th2), and TNF-<sub>1584 AD EPITOPE VACCINE</sub>

FIGURE 6. Expression of IL-18R (Th1) and T1/ST2 (Th2) molecules on the surface of CD4<sup>+</sup> T cells. Spleno-
cytes from all groups were depleted by CD8<sup>+</sup> T cells and restimulated with the same peptides that were used for in vivo injections, except splenocytes from mice immunized with Aβ<sub>35</sub> were activated in vitro with Aβ<sub>35</sub>. Splenocytes were analyzed before in vitro activation (0 day) and after 7 days of activation (7 day). For details, see Materials and Methods.

began an immunotherapy clinical trial on AD patients. Unfortunately, the phase II trial was halted because ~6% of the volunteers developed symptoms of an adverse inflammatory response in the brain (9, 17–20). It is unclear what was the actual cause of the adverse events in response to active immunization, but import-

antly, the Ab response to Aβ did not correlate with the presence or severity of the adverse events. In fact, some of the patients who developed meningoencephalitis did not have detectable levels of anti-Aβ Abs, suggesting that the adverse reaction to Aβ immunotherapy was not due to the humoral Ab response, but, rather, to a T cell-mediated immune response to AN-1792 (9, 20, 32, 53–56). The first case report on the neuropathology from an AD patient who was immunized multiple times with the AN-1792 vaccine supports the hypothesis that an adverse T cell-mediated autoimmune response was the cause of the aseptic meningoencephalitis (22). More specifically, neuropathological examination of the brain demonstrated infiltration of predominantly CD4<sup>+</sup> T cells in the leptomeninges and the cerebrovasculature in areas enriched with amyloid angiopathy. There were also sparse CD4<sup>+</sup> T cell infiltrates in the cerebral cortex and perivascular spaces. In addition, diffuse abnormalities in the cerebral white matter were observed, with a marked reduction in the density of myelinated fibers accompanied by extensive macrophage infiltration. However, there were also a number of promising changes in the neuropathology. Extensive areas of the neocortex were largely free of Aβ deposits, dystrophic neurits, and activated astrocytes, although these regions still contained neurofibrillary tangles, neuritil threads, and cere-

bral amyloid angiopathy. These results provide the first evidence that anti-Aβ Abs may significantly reduce Aβ pathology in AD patients. The second case report from the AN-1792 clinical trial (21) generally agrees with the first report, although there are some distinct differences, such as the presence of multinucleated giant cells filled with dense deposits of Aβ, multiple cortical hemorrhases, and CNS infiltration of CD8<sup>+</sup> T cells.

Drawing general conclusions regarding the effectiveness of Aβ immunotherapy from only two published case reports from the clinical trial is risky, because the brains of many elderly humans typically contain pathological lesions even though they may not present with clinical symptoms. However, there was additional preliminary evidence from the AN-1792 vaccine trial to suggest that Abs against Aβ may be beneficial to AD patients. For example, it was reported that some AD patients from the AN-1792 clinical trial showed significantly slower rates of decline in cogni-

tive functions and activities of daily living. The beneficial effects were also observed in two patients who suffered from transient attacks of meningoencephalitis. Importantly, the degree of protec-
tion was correlated with the titer of the Abs binding to Aβ plaques (19). Thus, the prominent T cell infiltration in the two case reports currently available from the clinical trial suggest that the aseptic subacute meningoencephalitis in patients who received the vaccine may have been caused by autoreactive anti-Aβ T cells, whereas the
We have designed a prototype AD vaccine that will induce Ab responses directed to the immunodominant B cell epitope of Aβ, but that will not generate anti-Aβ T cells. The immunodominant B cell epitope of Aβ1-15 has been mapped to the N terminus of this peptide (aa spanning residues 1–5, 1–7, 1–8, 1–11, 1–15, 1–16, or 4–10) (27–35). Based on these data, we chose the Aβ1-15 sequence as the B cell-immunodominant antigenic determinant for generation of our prototype epitope vaccine. Importantly, recent results clearly indicate that this Aβ1-15 peptide does not contain a T cell epitope in BALB/c mice (32) or in humans (57). The second component required for the design of the epitope vaccine was a T cell epitope that could provide strong T cell support to promote a potent humoral response to the Aβ1-15 B cell epitope in mice and humans. For our epitope vaccine we chose a universal synthetic, nonnatural pan HLA DR-binding epitope, PADRE, which was engineered to provide a Th cell epitope that is chemically defined, easily manufactured, and able to generate effective Th cell responses in the general human population. PADRE binds with high affinity to 15 of 16 of the most common HLA-DR types tested to date (37–43). When PADRE was tested against human T cells in a proliferation assay, it was found to be 100-fold more potent on a molar basis than a tetanus-derived universal epitope. PADRE also binds with high to intermediate affinity to mouse I-A^d and I-E^d MHC haplotypes (37, 39, 41, 42). In addition, PADRE has been shown to be safe and well tolerated in human clinical trials (40). We synthesized our epitope vaccine consisting of PADRE-Aβ1-15 on a MAP platform, which contains multiple copies of the antigenic determinant attached to a branched lysine backbone (44).

Mice immunized with our PADRE-Aβ1-15-MAP epitope vaccine formulated in alum, a Th2-type adjuvant, generated robust anti-Aβ32 humoral immune responses. The Ab titers were equivalent to titers in mice immunized with Aβ1-33-MAP, which contains both B and T cell epitopes of Aβ1-33 (Fig. 1). It is important to note that BALB/c mice respond very strongly to the T cell epitope of Aβ32 (32) and only moderately to the PADRE T cell epitope (39). Thus, even though BALB/c mice are low responders to PADRE, they still generated a strong anti-Aβ32 response. The Abs induced by PADRE-Aβ1-15-MAP were specific to Aβ, because they did not bind to the MAP backbone or PADRE (data not shown). Thus, the non-self T cell epitope PADRE promoted production of Abs against the Aβ1-15 B cell epitope and the anti-Aβ Abs bound to amyloid plaques in brain tissue from an AD patient. Ab isotyping has previously been used as an indirect measure of the contributions of Th1 (IgG2a) and Th2 (IgG1) cytokines to the disease: progress and problems on the road to therapeutics. Science 297:353.

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References


