PRELIMINARY COMMUNICATION

DNA β-Amyloid$_{1-42}$ Trimer Immunization for Alzheimer Disease in a Wild-Type Mouse Model

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Context DNA β-amyloid$_{1-42}$ (Aβ42) trimer immunization was developed to produce specific T helper 2 cell (T_H2)–type antibodies to provide an effective and safe therapy for Alzheimer disease (AD) by reducing elevated levels of Aβ42 peptide that occur in the brain of patients with AD.

Objective To compare the immune response in wild-type mice after immunization with DNA Aβ42 trimer and Aβ42 peptide.

Design and Intervention Wild-type mice received either 4 μg of DNA Aβ42 trimer immunization administered with gene gun (n=8) or intraperitoneal injection of 100 μg of human Aβ42 peptide with the adjuvant Quil A (n=8). Titers, epitope mapping, and isotypes of the Aβ42-specific antibodies were analyzed.

Main Outcome Measures Antibody titers, mapping of binding sites (epitopes), isotype profiles of the Aβ42-specific antibodies, and T-cell activation.

Results DNA Aβ42 trimer immunization resulted in antibody titers with a mean of 15 μg per milliliter of plasma. The isotype profile of the antibodies differed markedly. A predominant IgG1 antibody response was found in the DNA-immunized mice, indicating a T_H2 type of immune response (IgG1/IgG2a ratio of 10). The peptide-immunized mice showed a mixed T_H1/T_H2 immune response (IgG1/IgG2a ratio of 1) ($P<.001$). No increased T-cell proliferation was observed in the DNA-immunized mice ($P=.03$).

Conclusion In this preliminary study in a wild-type mouse model, DNA Aβ42 trimer immunization protocol produced a T_H2 immune response and appeared to have low potential to cause an inflammatory T-cell response.

JAMA. 2009;302(16):1796-1802 www.jama.com

ALZHEIMER DISEASE (AD) IS the most common cause of age-related dementia characterized by memory loss and cognitive decline. The pathogenesis of AD has been associated with the accumulation, aggregation, and deposition of β-amyloid (Aβ) peptides in the brain. Amyloid plaques and neurofibrillary tangles are both hallmarks of AD. The amyloid cascade hypothesis was formulated 15 years ago, postulating Aβ deposition as the initial event in the multifactorial pathogenesis of AD.[1-3] In triple-transgenic AD mouse models, it has been shown that Aβ assembly precedes tau pathology.[4,5]

The occurrence of familial forms of AD is linked to mutations in the *APP*, *PRES1*, and *PRES2* genes and all lead to overproduction of Aβ1-42.[1] Thus, the genetics of familial AD strongly supports the amyloid cascade hypothesis. Valuable mouse models of AD were developed by using mouse transgenics for the human familial AD genes, causing overproduction of human Aβ peptides and plaque development in mouse brain.[6]

There is significant evidence that Aβ peptides play a major role in onset and progression of AD.[7,8] Aggregated Aβ42 has been identified as a major component of senile plaques of AD brain and is thus a major target of therapy for AD. In mice, it has been shown that immunization with Aβ1-42 peptide leads to high titers of anti–Aβ1-42 antibodies and reduction of Aβ1-42 levels in the brain and therefore a reduction in total number of plaques in hippocampus and cortex.[9] In addition to these features, increased learning and memory have been described for immunized mice compared with respective control animals.[10,11]

A clinical trial in which patients with AD had been immunized with Aβ42 peptide was discontinued because meningoencephalitis occurred in 6% of immunized patients.[12,13] This may have been caused by the choice of QS21 as a T helper 1 cell (T_H1)–type of adjuvant.[14-16] Follow-up of a few study participants showed that Aβ42 peptide immunization did indeed lead to a reduction in plaque load in patients who had been treated with Aβ42 peptide.[17]

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To establish a safe AD immunization by shifting the immune response to T_H2 and avoiding inflammatory actions, we are developing genetic immunization as a method to reduce elevated brain levels of Aβ42 peptide, which contributes in part to the dementia in patients with AD.[18-20] T_H2 cells are a subset of lymphocytes that produce specific cytokines that program B cells to synthesize specific antibodies that have low probability to cause inflammation. Conversely, T_H1 cells are another subset of lymphocytes that program B cells to synthesize a different set of antibodies that have a high probability to cause inflammation.

We have shown that DNA Aβ42 immunization produces a T_H2 immune response.[18-20] This point is important because Aβ42 peptide immunization in a prior clinical trial produced a T_H1 immune response that is likely to produce inflammation.[12-16] Our original findings recently have been confirmed and extended by DaSilva et al,[21] in which using Aβ42 DNA immunization produced high levels of Aβ42-specific T_H2 type antibodies and resulted in significant reduction of plaques in a transgenic mouse model.

In this study, the immune response is compared in wild-type mice after immunization with DNA Aβ42 trimer and with Aβ42 peptide. Titers, epitope mapping, and isotypes of the Aβ42-specific antibodies were analyzed. These studies were designed to determine the effectiveness and safety of DNA Aβ42 trimer immunization as a potential therapy for AD.

METHODS

Animals

All experiments were performed in 4- to 8-month-old female B6SJLF1/J mice (Jackson Laboratory, Bar Harbor, Maine). Animal use for this study was approved by the University of Texas Southwestern Medical Center animal research committee. Eight mice received DNA Aβ42 trimer immunization; 8 mice were immunized with human Aβ42 peptide.

DNA Constructs

For DNA immunization, a new construct was developed to increase Aβ42 expression.[22] This system consists of 2 plasmids, an activator plasmid encoding the yeast GAL4 transcription factor and a responder plasmid encoding the gene for which expression is driven by binding of GAL4 to UAS (upstream activating sequences) sites upstream of a minimal promoter.[23] The open reading frame for Aβ42 was synthesized 3 times in a row and cloned into a responder immunization vector downstream of a UAS promoter (FIGURE 1). Aβ42-trimer/UAS responder plasmid and GAL4 activator plasmid were mixed in a 5:1 ratio for preparation of the DNA-coated gold particles.

Peptides

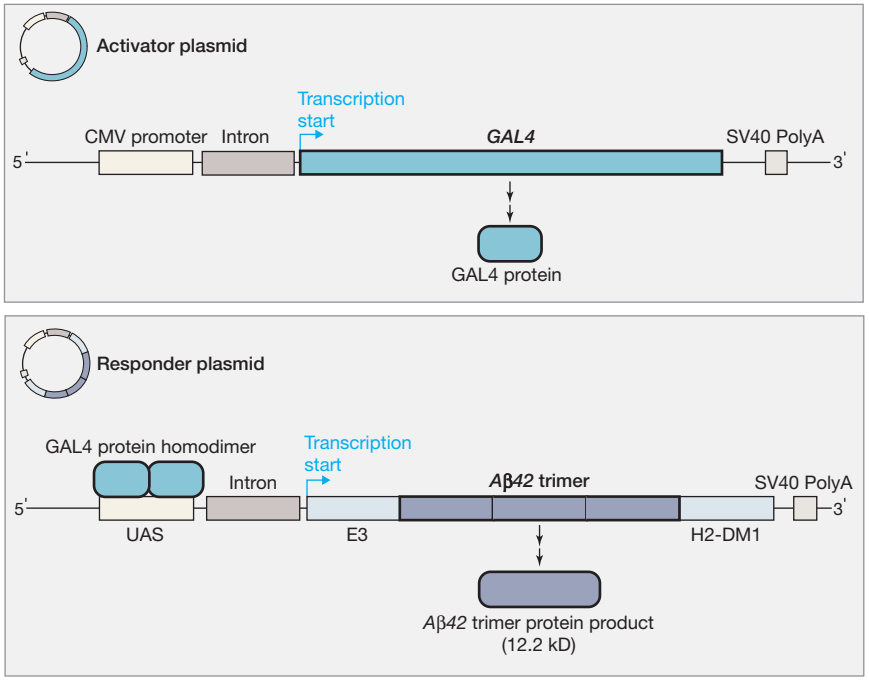
Human Aβ1-42 peptide was obtained from rPeptide (Bogart, Georgia). Custom Aβ42 peptides (Aβ1-15, Aβ6-20, Aβ10-25, Aβ16-30, and Aβ21-35) were purchased from JPT Peptide Technologies (Berlin, Germany). Other, shorter Aβ peptides, Aβ1-9, Aβ4-10, Aβ5-14, and mouse Aβ1-16, were purchased from AnaSpec (San Jose, California).

Immunizations

Immunizations with plasmid DNA encoding the Aβ42 trimer were performed on ear skin using the Helios gene gun (Bio-Rad Laboratories, Hercules, California) as described previously.[22] DNA-coated gold particles were injected into both sides of the ears with a helium gas pressure of 400 psi. Each immunization consisted of a total of 4-μg DNA.

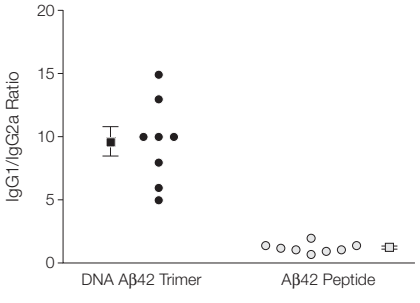
Human Aβ1-42 peptide was dissolved directly in phosphate-buffered saline (PBS) at 4 mg/mL and incubated overnight at 37°C, allowing fibril formations.[24] The solution was diluted in PBS to a concentration of

Figure 1. Schematic Representation of the Double Plasmid System (GAL4 Activator, UAS/Aβ42 Trimer Responder) Used for Genetic Immunization



Constitutive expression of the GAL4 transcription factor is driven by a cytomegalovirus (CMV) promoter on the activator plasmid. The GAL4 protein binds as a homodimer to the responder plasmid at sites in the upstream activator sequence (UAS), part of a minimal promoter. GAL4 binding enhances transcription of the β-amyloid$_{1-42}$ (Aβ42) trimer sequence that has been cloned into a DNA fragment between an adenovirus E3 (early region 3) leader sequence and an endosomal targeting sequence derived from the mouse major histocompatibility complex class II gene *H2-DM*. These flanking regions are involved in targeting the messenger RNA (mRNA) transcript to the endoplasmic reticulum for protein synthesis and secretion. The simian virus 40 (SV40) polyadenylation (PolyA) sequence on both the activation and responder plasmids stabilize mRNA transcripts.

Figure 2. Different IgG Isotypes in B6SJLF1/J Mice After Peptide and DNA Immunization With Human β-Amyloid$_{1-42}$



From a blood sample taken after 4 vaccinations, the ratio of the optical density values of IgG1 to IgG2a was calculated. Mice immunized with DNA (D1-D8) showed a predominant IgG1 response indicating a T helper 2 cell (T_H2) response (mean [SD] IgG1/IgG2a ratio, 9.63 [1.18]). Mice immunized with peptide (P1-P8) showed both isotypes, IgG1 and IgG2a, indicating a mixed T_H2/T_H1 response (mean [SD] IgG1/IgG2a ratio, 1.23 [0.14]). $P < .001$ for comparison between the groups. Square data markers indicate group means; error bars indicate SD.

2 mg/mL. Aliquots were stored at −20°C. For all peptide immunizations, Aβ (100 µg per mouse per injection) and the adjuvant Quil A (20 µg per mouse per injection; Sigma, St Louis, Missouri) were mixed just prior to intraperitoneal immunization.

Blood samples were collected 7 days after each immunization procedure and analyzed for antibody titers and isotype profiles. The immunizations were performed in 2 sets of experiments with 8 mice in each, with 4 mice receiving DNA immunizations and 4 mice receiving peptide immunizations. In the first set, all mice received a total of 6 immunizations; in the second set, 4 mice received 6 peptide immunizations and 4 mice received 8 DNA immunizations in biweekly intervals.

Plasma Collection and Tissue Harvest

Submandibular blood samples were collected 7 days after each immunization and the plasma frozen at −20°C. Mice were killed 10 days following the final immunization. Blood was collected by cardiac puncture and immediately mixed with 10 µL of 0.2-M EDTA. The spleen was aseptically removed and used for tissue culture.

Anti-Aβ Antibody Enzyme-Linked Immunosorbent Assay

Anti-Aβ antibodies in mouse plasma were measured according to standard procedures. Round-bottom 96-well plates were coated with Aβ1-42 (2 µg/mL) in 50-mM carbonate buffer (pH 9.6) overnight at 4°C. Standard curves were included by adding serial dilutions of the mouse anti-Aβ monoclonal antibody 4G8 in PBS with 1.5% bovine serum albumin or serial dilutions of murine IgG or IgM. Plasma samples were diluted in PBS at concentrations ranging from 1:200 to 1:2000 and incubated overnight at 4°C. Bound antibody was detected using horseradish peroxidase (HRP)–conjugated goat anti-mouse IgG or IgM and developed with 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) substrate. For epitope mapping, plates were coated with the shorter Aβ42 peptides. To determine antibody isotypes, a second antibody incubation step was included in the enzyme-linked immunosorbent assay (ELISA) protocol using isotype-specific biotinylated antibodies (IgG1 and IgG2a^b [BD Biosciences, San Jose]), which were detected with HRP-labeled streptavidin (BD Biosciences). The IgG1/IgG2a ratio was calculated for each mouse individually using the optical density values obtained from the isotype-specific ELISA.

Splenocyte Proliferation Assay

Cells were plated in round-bottom 96-well plates at 5×10^6 cells/mL in complete Royal Park Memorial Institute medium 1640 (BioWhittaker, Walkersville, Maryland). Aβ42 peptides were diluted in complete Royal Park Memorial Institute medium 1640 and added into the cultures. At 48, 72, 96, and 120 hours, supernatants were collected and frozen at −20°C until cytokine analysis. To measure proliferation, 1 µCi of tritium-labeled thymidine was added at 72 hours. Eighteen hours later, cells were harvested and thymidine incorporation determined using a liquid scintillation counter. A stimulation index was calculated using the following formula: counts per minute (CPM) of wells with Aβ42 antigen divided by CPM of wells with no antigen.

Cytokine ELISA

Matching antibody pairs for murine IL-2, IL-4, IL-5, IL-10, IL-13, interferon γ (IFN-γ), and tumor necrosis factor (TNF) (eBioscience, San Diego, California) were used according to the manufacturer's instructions. Serial cytokine dilutions were added as standards and supernatants from Concanavalin A (ConA) stimulated splenocytes were included as positive controls. Splenocyte supernatants were added to the plate in a 1:1 dilution with medium. ConA supernatants were used in a 1:20 dilution. Plasma was used in a 1:4 dilution. Antibody binding was detected by tetramethylbenzidine (TMB) substrate, and after stop buffer was added, the plates were read at 450 nm. Cytokine ELISAs from cell culture supernatants were done in quadruplicates; cytokine ELISAs from mouse plasma were performed in duplicates.

Statistics

Statistical analysis (1-way analysis of variance, column statistics, unpaired *t* test with 2-tailed *P* values, and 95% confidence intervals) were performed using GraphPad Prism version 5.02 and GraphPad StatMate version 2 for Windows (GraphPad Software, San Diego, California).

RESULTS

Generation of Anti-Aβ Antibody Response in B6SJLF1/J Mice

To analyze the humoral immune response against Aβ42 in B6SJLF1/J mice, blood plasma was collected 1 week after each immunization, and anti-Aβ antibody levels were determined by ELISA. Anti-Aβ42 IgG antibodies were not detected in preimmune plasma from any mouse used here. After the first immunization, low levels of anti-Aβ antibodies could be detected in 7 of 8 animals given the DNA gene vaccine mainly of the IgM isotype and in all mice given the peptide vaccine. The mice receiving peptide immunizations reached mean anti-Aβ1-42 IgG antibody values of 500 µg per milliliter of plasma after 4 immunizations (n=8) and 1.2 mg/mL (SD, 300 µg/mL)

after 6 immunizations. The IgG antibody titer in the DNA-immunized mice (n=8) increased to a mean of 3 µg/mL after 4 immunization time points and had a mean (SD) of 10 (5) µg/mL after 6 immunizations. Due to this difference in antibody levels between the 2 groups, half of the mice immunized with DNA received 8 booster immunizations (n=4, D5-D8), which led to a mean (SD) antibody titer of 15 (15) µg per milliliter of plasma, most likely an effective immune response.

Isotype Profiles of Humoral Immune Response

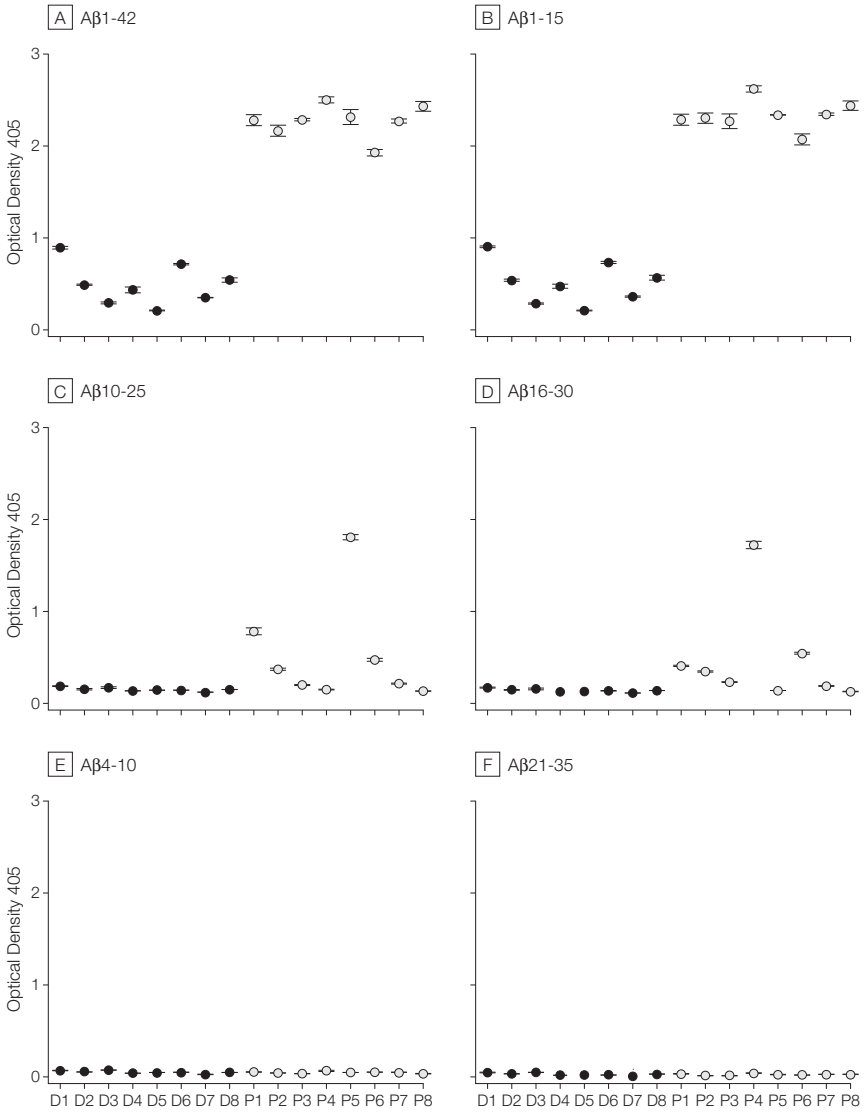
The subclass of immunoglobulin induced after immunization is an indirect measure of the relative contribution of T_H1-type cytokines vs T_H2-type cytokines. The production of IgG1 antibodies is primarily induced by IL-4, which is a T_H2 cytokine, whereas production of IgG2 antibodies indicates the involvement of T_H1-type cytokines such as IFN-γ.[25]

Therefore, as an indicator of the T_H1 vs T_H2 profile of the humoral immune response, the ratio of the optical density values of IgG1 to IgG2a was calculated and the mean value was compared between the different groups for the blood sample taken after 4 vaccinations (FIGURE 2). Mice immunized with DNA (n=8) showed a predominant IgG1 response indicating a T_H2 response with a mean IgG1/IgG2a ratio of 10 in the DNA-immunized mice, while peptide-immunized mice (n=8) had an IgG/IgG2a ratio of approximately 1, indicating a mixed T_H1/T_H2 response. The 95% confidence interval in this analysis was 5.85 to 10.94; the means were significantly different ($P<.001$).

Mapping of Antibody Epitopes

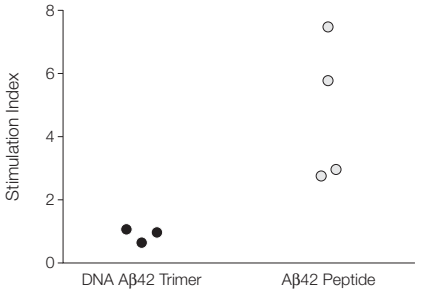
To further characterize the humoral response, epitope mapping (the positions or amino acid regions on the Aβ42 peptide) of Aβ antibody binding was performed using overlapping peptides of Aβ42. The main B-cell epitope is known to be located in the N-terminal regions of Aβ42. The selection of 5 overlapping peptides covered most of the human Aβ1-42 sequence, amino acids 1-15, 6-20, 10-25, 16-30, and 21-35. In addition, we used human Aβ peptides 1-9, 4-10, and 5-14, as well as a peptide for the mouse Aβ1-42 B-cell epitope, Aβ1-16, carrying the 3 amino acids differentiating human and mouse Aβ. Aβ42-1 was used as a negative control. The IgG antibody response was directed against human Aβ1-15, which is the known B-cell epitope. No reactivity was found against Aβ6-20, Aβ21-35, Aβ1-9, Aβ4-10, and Aβ5-14; some antibodies in the antiserum samples from the peptide-immunized mice bound to Aβ10-25 and Aβ16-30 (FIGURE 3).

Figure 3. Epitope Mapping of the β-Amyloid$_{1-42}$ Antibodies in Mice Immunized With DNA Aβ1-42 Trimer and Aβ1-42 Peptide



A similar pattern of antibody binding sites (epitopes) was found for DNA-immunized mice (D1 to D8) and peptide-immunized mice (P1 to P8). All plasma samples were used in a 1:500 dilution and were analyzed in triplicates. Positive binding was found for full-length Aβ1-42 and Aβ1-15 in DNA- and peptide-immunized plasma samples (A and B). Some plasma samples from the peptide-immunized mice had high titers of antibodies binding to different epitopes, Aβ10-25 and Aβ16-30 (C and D). No binding was found for Aβ4-10 and Aβ21-35 (E and F), as well as for Aβ1-9, Aβ5-14, and Aβ6-20 (data not shown). Each data marker represents a mean of 3 values; error bars indicate standard deviation.

Figure 4. Stimulation Indexes for β-Amyloid$_{42}$ Restimulated Splenocyte Cultures



Cells were cultured and restimulated with Aβ1-42 peptide (10 ug/mL) 10 days after the final immunization. Incorporation of tritium-labeled thymidine was used to measure cell proliferation and a stimulation index (SI) was calculated using the following formula: counts per minute (CPM) of wells with Aβ42 antigen divided by CPM of wells with no antigen. DNA-immunized mice had an SI of less than 1 (mean [SD], 0.93 [0.12]; n=3) and peptide-immunized mice had an SI of 2 to 7 (mean [SD], 4.77 [1.14]; n=4). $P=.03$ for comparision between the groups.

Strong cross-reactivity between mouse and human Aβ1-42 was detected in the peptide-immunized mice. This reactivity was directed primarily at mouse Aβ1-16, which contains all of the amino acid differences between human and mouse, positions 5, 10, and 13 (data not shown).

Aβ1-42 T-Cell Response

To characterize the cellular immune response induced by the different Aβ42 immunization protocols, splenocyte cultures were generated from individual animals 10 days after the final immunization, and the cultures were restimulated with different concentrations of Aβ42 peptide in vitro for 5 days. To ensure cell viability, splenocytes from each mouse were also stimulated with ConA or anti-CD3 antibody. While the peptide-immunized mice showed stimulation indexes between 2 and 7 for Aβ42 peptide restimulation, no increased proliferation was observed in the DNA-immunized mice restimulated with the Aβ42 peptide (FIGURE 4). The 95% confidence interval in this analysis was –7.31 to –0.37 ($P=.03$).

Next, we sought to determine the effects of DNA and peptide immunization on T effector cell differentiation by analyzing cytokine expression (IL-2, IL-4, IL-5, IL-10, IL-13, IFN-γ, and TNF) by ELISA. Consistent with the T-cell proliferation results, the mice vaccinated with Aβ1-42 peptide developed much higher levels of cytokine expression in response to Aβ1-42 peptide restimulation than DNA Aβ42 trimer–immunized mice. Mean (SD) IL-2 production was different between splenocytes from peptide (300 [50] pg/mL) and DNA-immunized mice (25 [10] pg/ml) ($P<.001$). Mean (SD) levels of the T_H1 cytokine IFN-γ also showed changes between peptide- and DNA-vaccinated groups, (250 [100] pg/mL vs 20 [10] pg/mL, respectively; $P=.02$). The T_H2 cytokine IL-13 had mean (SD) levels of 30 [10] pg/mL in supernatant from DNA-immunized mice and reached more than 1000 [200] pg/mL ($P=.001$) in the supernatant from peptide-immunized mice. Also for IL-5, the Aβ trimer DNA–vaccinated mice had 15 to 30 pg/mL in the supernatant from restimulated splenocytes while the Aβ42 peptide–immunized mice had mean (SD) levels of 500 [200] pg/mL ($P=.01$). The cytokine IL-10 was detectable in the restimulated cultures only from 1 DNA-vaccinated mouse with levels of 1000 pg/mL. Cytokines TNF and IL-4 were below detection levels. The lack of IL-4 in these Aβ42 peptide restimulated cultures was due to the mouse strain used, B6SJLF1/J. The strains C57BL6 and SJL are both low IL-4 producers.[26] ConA and anti-CD3 supernatants from all mice tested contained more than 15 000 pg/mL of IFN-γ and more than 800 pg/mL of IL-2.

To confirm the IL-10 presence in the restimulated splenocyte culture of DNA-vaccinated mice, the IL-10 levels in plasma were analyzed. Although high levels of IL-10 (500-2000 pg/mL) were found in plasma in 4 of 5 tested samples from the final blood samples from the DNA-immunized mice, similar levels were also found in 2 of 7 samples tested for the peptide-immunized mice, probably because of the mixed T_H1/T_H2 response.

COMMENT

Alzheimer disease is a neurodegenerative disorder for which immunotherapy has treatment potential using either active or passive Aβ42 immunizations to reduce Aβ42 peptide levels in the brains of patients with AD.[27]

To verify that DNA immunization is a safe way to proceed in the treatment of AD patients, results from a direct comparison of the gene gun–mediated DNA Aβ42 immunization in mice were compared with the results from mice that had been immunized with Aβ42 peptide and Quil A adjuvant. The immune response was assessed 2 ways. First, we analyzed the humoral response in the 2 groups of mice. DNA immunization with the double plasmid system produced a mean antibody titer of 15 µg/mL, which was much less when compared with peptide immunization (1.2 mg/mL) but significantly increased compared with previous studies using an Aβ42 monomer (1-copy) plasmid.[18-20] Although antibody levels derived from DNA immunization were lower than levels produced by peptide immunization, the achieved levels are most likely effective, and the antibody isotype is biased to T_H2, indicating a low probability to cause inflammation. A predominant T_H2 response is a more important objective in developing an effective and safe therapy for AD than increased antibody levels alone.

The antibody isotypes obtained are a direct reflection of the cytokine production during an immune response. T_H1 cytokines like IFN-γ promote immunoglobulin class switching from IgM to IgG2a, whereas T_H2 cytokines like IL-4 and IL-5 lead to immunoglobulin isotype switching to IgG1.[25,28] A predominant IgG1 antibody response (T_H2 type) in the DNA-immunized mice and a mixed IgG1/IgG2a (T_H2/T_H1) response after peptide vaccination is consistent with results published from other groups that show that Aβ42 DNA vaccination leads indeed to a polarized T_H2 response.[29-31] Also in the peptide immunization protocols, it is well established that the type of adjuvant

used has a major influence on the elicited immune response.[32,33] Qs21, a more highly refined derivative of Quil A and a known T_H1-type adjuvant, was used in the clinical trial.[12-16,33] Immunization with Aβ1-42 peptide and Quil A yielded a broad cross-reactive IgG1/IgG2a immune response as compared with the solely IgG1 isotype (T_H2 type) in the DNA Aβ42 trimer–immunized mice.

The binding sites (epitopes) of Aβ42 antibodies analyzed in an AD mouse model showed that antibodies directed against an N-terminal region (epitope), amino acids 1-15 of the 42 amino acids of the peptide (Aβ1-15), were more effective in plaque clearance than antibodies detecting C-terminal epitopes,[34] which is consistent with the antibody binding site (epitope) mappings for DNA and peptide immunization at Aβ1-15 in this study. In the same study,[34] the authors also showed an important impact of the antibody isotypes: IgG2a antibodies were the most effective antibodies to reduce Aβ42 levels, likely through their high affinity for Fc receptor binding (FcγRI) on phagocytic microglia cells. However, it is also believed that T_H1-type antibodies contributed to the inflammation in the clinical trial.[12,13] In a different study, it was shown that the highest activity levels for reduction of Aβ42 levels by microglia cultures were found for IgG1 antibodies (T_H2 type).[35]

Several antibody actions that result in reducing brain levels of Aβ42 peptide are implicated, including the opsonization of Aβ42 to increase phagocytosis, the interference of antibodies with Aβ aggregation, and the function of antibodies as a peripheral sink without entering the brain.[36] The observed broader reactivity of the antibodies from peptide-immunized mice is a characteristic feature of a T_H1 immune response. In some respects, the breadth of the immune responses, an increased number of loci or epitopes of antibody binding to the Aβ42 peptide seen following peptide immunization, is analogous to the process of epitope spreading (antibody binding to increased number of regions on a particular peptide), seen in the SJL mouse model of experimental autoimmune encephalomyelitis, a mouse model for multiple sclerosis. In that model, epitope spreading is associated with disease worsening and progression.[37] Thus, the highly focused and T_H2 predominant response seen following DNA immunization indicates a reduced risk for inflammation in AD patients.

Analyzing the T-cell response in both the DNA Aβ42– and peptide-immunized mice was essential in pursuing an immunization approach for AD treatment. Circulating Aβ-reactive T cells are present in patients with AD without any previous immunizations.[14] In the experiment, the T-cell reactivity, response of T cells to Aβ42 peptide in cell culture, was very low in the DNA-immunized mice. However, it is clear that T cells had been involved earlier in the immunization procedure and is demonstrated by the switching of the immunoglobulin isotypes from IgM to IgG1 (T_H2 help). The lack of Aβ42-reactive T cells after their initial help in B-cell activation and respective antibody production might be favorable in regard to the immunization of patients with AD by this method, because without Aβ42-specific T cells (and lack of IFN-γ), the risk of inflammation is low. Different from other DNA Aβ42 immunization approaches in which only parts of the Aβ peptide were included,[31,38-40] we used full-length DNA Aβ42 trimer containing both B- and T-cell epitopes and still were able to show nonreactive T cells to restimulation with Aβ42 peptide, again indicating a low probability of an inflammatory T-cell response when administered to patients with AD. Further detailed analysis of the suggested exhaustion of a specific T-cell response after DNA Aβ42 trimer immunization is of high interest.

Finding the cytokine IL-10 in DNA-immunized mice is of particular interest, since IL-10 is described as an anti-inflammatory cytokine that down-regulates T_H1 cells.[41,42] IL-10 up-regulation (4000 pg per milliliter of plasma) has been shown in mice immunized with an 11x-Aβ1-6 DNA adenovirus vector, and Kim et al[43] suggested that IL-10 production drives the T_H2 response in their immunization protocol. Since this particular cytokine was found in the DNA-immunized mice, further analysis of IL-10 is indicated.

The parallel comparison of gene gun–delivered DNA Aβ42 immunization and intraperitoneal injections of Aβ42 peptide showed the characteristic features of genetic immunizations. DNA Aβ42 trimer immunization of wild-type mice resulted in a strongly polarized T_H2 immune response that has high probability to be noninflammatory and therefore safe as therapy to delay AD.

If DNA Aβ42 trimer immunization is able to reduce Aβ42 peptide levels in the brains of patients with AD by one-half as it does in the transgenic mouse brain,[16-21] it may be possible to slow progression of AD for several years. The overall potential of DNA Aβ42 immunization may be considerable, and effort should be undertaken to test it thoroughly.

Author Contributions: Dr Lambracht-Washington had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Drs Lambracht-Washington and Qu contributed equally to the work presented in this article.
Study concept and design: Lambracht-Washington, Qu, Eagar, Stüve, Rosenberg.
Acquisition of data: Lambracht-Washington, Qu, Fu.
Analysis and interpretation of data: Lambracht-Washington, Eagar, Stüve, Rosenberg.
Drafting of the manuscript: Lambracht-Washington, Qu, Fu, Eagar, Stüve, Rosenberg.
Critical revision of the manuscript for important intellectual content: Lambracht-Washington, Eagar, Stüve, Rosenberg.
Statistical analysis: Lambracht-Washington.
Obtained funding: Rosenberg.
Administrative, technical, or material support: Lambracht-Washington, Qu, Fu, Eagar, Stüve.
Study supervision: Rosenberg.
Financial Disclosures: Dr Rosenberg reported receiving clinical trial grants from Elan and Pfizer; being listed as a coinventor on US patent application for "Abeta gene vaccines," January 2009; and being assigned to the Regents of the University of Texas system. No other disclosures were reported.
Funding/Support: This study was supported by the University of Texas Southwestern Alzheimer's Disease Center, grant P30AG12300-16 from the National Institutes of Health National Institute on Aging, the Rudman Foundation, and research grant IIRG-06-24428 from the Alzheimer's Association.
Role of the Sponsor: The funding organizations had no role in the design and conduct of the study; in the

collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

Disclaimer: Dr Rosenberg, an editorial board member for *JAMA*, was not involved in the editorial review of or decision to publish this article.

REFERENCES

1. Hardy J. New insights into the genetics of Alzheimer's disease. *Ann Med*. 1996;28(3):255-258.

2. Selkoe DJ. Amyloid beta-protein and the genetics of Alzheimer's disease. *J Biol Chem*. 1996;271 (31):18295-18298.

3. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*. 2002;297(5580):353-356.

4. Götz J, Chen F, van Dorpe J, Nitsch RM. Formation of neurofibrillary tangles in P301L tau transgenic mice induced by Abeta 42 fibrils. *Science*. 2001; 293(5534):1491-1495.

5. Oddo S, Caccamo A, Kitazawa M, Tseng BP, LaFerla FM. Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. *Neurobiol Aging*. 2003;24(8):1063-1070.

6. Dodart JC, Mathis C, Bales KR, Paul SM. Does my mouse have Alzheimer's disease? *Genes Brain Behav*. 2002;1(3):142-155.

7. Bertram L, Tanzi RE. The current status of Alzheimer's disease genetics: what do we tell the patients? *Pharmacol Res*. 2004;50(4):385-396.

8. Rosenberg RN. Translational research on the way to effective therapy for Alzheimer's disease. *Arch Gen Psychiatry*. 2005;62(11):1186-1192.

9. Schenk D, Barbour R, Dunn W, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature*. 1999; 400(6740):173-177.

10. Janus C, Pearson J, McLaurin J, et al. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature*. 2000;408(6815):979-982.

11. Morgan D, Diamond DM, Gottschall PE, et al. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature*. 2000; 408(6815):982-985.

12. Fox NC, Black RS, Gilman S, et al; AN1792(QS-21)-201 Study. Effects of Abeta immunization (AN1792) on MRI measures of cerebral volume in Alzheimer disease. *Neurology*. 2005;64(9):1563-1572.

13. Gilman S, Koller M, Black RS, et al; AN1792(QS-21)-201 Study Team. Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. *Neurology*. 2005;64(9):1553-1562.

14. Monsonego A, Zota V, Karni A, et al. Increased T cell reactivity to amyloid beta protein in older humans and patients with Alzheimer disease. *J Clin Invest*. 2003;112(3):415-422.

15. Monsonego A, Weiner HL. Immunotherapeutic approaches to Alzheimer's disease. *Science*. 2003; 302(5646):834-838.

16. Monsonego A, Imitola J, Petrovic S, et al. Abeta-induced meningoencephalitis is IFN-gamma-dependent and is associated with T cell-dependent clearance of Abeta in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2006;103(13): 5048-5053.

17. Holmes C, Boche D, Wilkinson D, et al. Long-term effects of Abeta42 immunization in Alzheimer's disease: follow-up of a randomized, placebo-controlled phase I trial. *Lancet*. 2008;372(9634): 216-223.

18. Qu B, Rosenberg RN, Li L, Boyer PJ, Johnston SA. Gene vaccination to bias the immune response to amyloid-beta peptide as therapy for Alzheimer disease. *Arch Neurol*. 2004;61(12):1859-1864.

19. Qu B, Boyer PJ, Johnston SA, Hynan LS, Rosenberg RNA. β42 gene vaccination reduces brain amyloid plaque burden in transgenic mice. *J Neurol Sci*. 2006; 244(1-2):151-158.

20. Qu BX, Xiang Q, Li L, Johnston SA, Hynan LS, Rosenberg RNA. β42 gene vaccine prevents Aβ42 deposition in brain of double transgenic mice *J Neurol Sci*. 2007;260(1-2):204-213.

21. DaSilva KA, Brown ME, McLaurin J. Reduced oligomeric and vascular amyloid-beta following immunization of TgCRND8 mice with an Alzheimer's DNA vaccine. *Vaccine*. 2009;27(9):1365-1376.

22. Qu BX, Sinclair D, Fu M, Lambracht-Washington D, Rosenberg RN. Trimer Aβ42 gene vaccine elicits a high level of antibody production when delivered with the GAL4-UAS system and the gene gun. *Alzheimer Dement*. 2008;4(4)(suppl 2):T477.

23. Ornitz DM, Moreadith RW, Leder P. Binary system for regulating transgene expression in mice: targeting int-2 gene expression with yeast Gal4/UAS control elements. *Proc Natl Acad Sci U S A*. 1991; 88(3):698-702.

24. Walsh DM, Lomakin A, Benedek GB, Condron MM, Teplow DB. Amyloid beta-protein fibrillogenesis: detection of a protofibrillar intermediate. *J Biol Chem*. 1997;272(35):22364-22372.

25. Finkelman FD, Holmes J, Katona IM, et al. Lymphokine control of in vivo immunoglobulin isotype selection. *Annu Rev Immunol*. 1990;8:303-333.

26. Choi P, Xanthaki D, Rose SJ, Haywood M, Reiser H, Morley BJ. Linkage analysis of the genetic determinants of T-cell IL-4 secretion, and identification of Flj20274 as a putative candidate gene. *Genes Immun*. 2005;6(4):290-297.

27. Foster JK, Verdile G, Bates KA, Martins RN. Immunization in Alzheimer's disease: naïve hope or realistic clinical potential? *Mol Psychiatry*. 2009; 14(3):239-251.

28. Erickson LD, Foy TM, Waldschmidt TJ. Murine B1 B cells require IL-5 for optimal T cell-dependent activation. *J Immunol*. 2001;166(3):1531-1539.

29. Kim HD, Jin JJ, Maxwell JA, Fukuchi K. Enhancing Th2 immune responses against amyloid protein by a DNA prime-adenovirus boost regimen for Alzheimer's disease. *Immunol Lett*. 2007;112(1):30-38.

30. Frazer ME, Hughes JE, Mastrangelo MA, Tibbens JL, Federoff HJ, Bowers WJ. Reduced pathology and improved behavioral performance in Alzheimer's disease mice vaccinated with HSV amplicons expressing amyloid-beta and interleukin-4. *Mol Ther*. 2008; 16(5):845-853.

31. Movsesyan N, Ghochikyan A, Mkrtichyan M, et al. Reducing AD-like pathology in 3xTg-AD mouse model by DNA epitope vaccine: a novel immunotherapeutic strategy. *PLoS One*. 2008;3(5):e2124.

32. Cribbs DH, Ghochikyan A, Vasilevko V, et al. Adjuvant-dependent modulation of Th1 and Th2 responses to immunization with beta-amyloid. *Int Immunol*. 2003;15(4):505-514.

33. Ghochikyan A, Mkrtichyan M, Petrushina I, et al. Prototype Alzheimer's disease epitope vaccine induced strong Th2-type anti-Abeta antibody response with Alum to Quil A adjuvant switch. *Vaccine*. 2006;24(13):2275-2282.

34. Bard F, Barbour R, Cannon C, et al. Epitope and isotype specificities of antibodies to beta-amyloid peptide for protection against Alzheimer's disease-like neuropathology. *Proc Natl Acad Sci U S A*. 2003; 100(4):2023-2028.

35. Mohajeri MH, Gaugler MN, Martinez J, et al. Assessment of the bioactivity of antibodies against beta-amyloid peptide in vitro and in vivo. *Neurodegener Dis*. 2004;1(4-5):160-167.

36. Holtzman DM, Bales KR, Paul SM, DeMattos RB. Abeta immunization and anti-Abeta antibodies: potential therapies for the prevention and treatment of Alzheimer's disease. *Adv Drug Deliv Rev*. 2002; 54(12):1603-1613.

37. Vanderlugt CL, Begolka WS, Neville KL, et al. The functional significance of epitope spreading and its regulation by co-stimulatory molecules. *Immunol Rev*. 1998;164:63-72.

38. Lemere CA, Maier M, Peng Y, Jiang L, Seabrook TJ. Novel Abeta immunogens: is shorter better? *Curr Alzheimer Res*. 2007;4(4):427-436.

39. Maier M, Seabrook TJ, Lazo ND, et al. Short amyloid-beta (Abeta) immunogens reduce cerebral Abeta load and learning deficits in an Alzheimer's disease mouse model in the absence of an Abeta-specific cellular immune response. *J Neurosci*. 2006;26(18): 4717-4728.

40. Zou J, Yao Z, Zhang G, et al. Vaccination of Alzheimer's model mice with adenovirus vector containing quadrivalent foldable Aβ1-15 reduces Aβ burden and behavioral impairment without Aβ-specific T cell response. *J Neurol Sci*. 2008;272(1-2):87-98.

41. Akdis CA, Blaser K. Mechanisms of interleukin-10-mediated immune suppression. *Immunology*. 2001; 103(2):131-136.

42. O'Garra A, Vieira P. T(H)1 cells control themselves by producing interleukin-10. *Nat Rev Immunol*. 2007;7(6):425-428.

43. Kim HD, Tahara K, Maxwell JA, et al. Nasal inoculation of an adenovirus vector encoding 11 tandem repeats of Abeta1-6 upregulates IL-10 expression and reduces amyloid load in a Mo/Hu APPswe PS1dE9 mouse model of Alzheimer's disease. *J Gene Med*. 2007;9(2):88-98.

