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ORIGINAL ARTICLE

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Antibodies from a DNA peptide vaccination decrease the brain amyloid burden in a mouse model of Alzheimer's disease

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Abstract The neuropathology of Alzheimer's disease (AD) is characterized by the accumulation of amyloid peptide Aβ in the brain derived from proteolytic cleavage of the amyloid precursor protein (APP). Vaccination of mice with plasmid DNA coding for the human Aβ_{42} peptide together with low doses of preaggregated peptide induced antibodies with detectable titers after only 2 weeks. One serum was directed against the four amino-terminal amino acids DAEF and differs from previously described ones. Both immune sera and monoclonal antibodies solubilized preformed aggregates of Aβ_{42} in vitro and recognized amyloid plaques in brain sections of mice transgenic for human APP. Passive immunization of transgenic AD mice caused a significant and rapid reduction in brain amyloid plaques within 24 h. The combined DNA peptide vaccine may prove useful for active immunization with few inoculations and low peptide dose which may prevent the recently described inflammatory reactions in patients. The monoclonal antibodies are applicable for passive immunization studies and may lead to a therapy of AD.

Keywords Alzheimer's disease · DNA vaccine · Monoclonal antibodies

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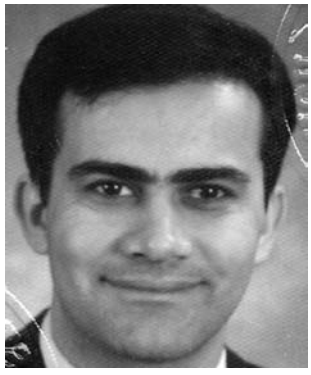

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Abbreviations *AD:* Alzheimer's disease · *Aβ:* Amyloid-β · *APP:* Amyloid precursor protein · *ELISA:* Enzyme-linked immunosorbent assay · *GM-CSF:* Granulocyte-macrophage colony-stimulating factor · *IL:* Interleukin · *SDS:* Sodium dodecyl sulfate · *tPA:* Tissue-type plasminogen activator

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder which is preceded by the deposition of amyloid-β (Aβ) peptides into Aβ plaques and brain blood vessel walls [1]. The Aβ peptides are 39–43 amino acids in length and derived from sequential proteolytic cleavage of the amyloid precursor protein (APP) [2]. APP is a large membrane protein expressed ubiquitously throughout many tissues including brain. Aβ peptides convert from soluble molecules to insoluble aggregates in a concentration-dependent manner. These amyloid aggregates are formed from a soluble, mainly coiled, and slightly α-helical form and are characterized by a high degree of β-sheets, a fibrillar morphology in electron microscopy, and the ability to bind and alter spectroscopic properties of thioflavin [3]. Recently evidence has accumulated which suggests that AD is a synaptic failure due to diffusible oligomers of amyloid peptides [4].

A mouse model of brain amyloid plaque formation expresses the human APP gene with a familial AD mutation (APP V717F) under the control of a neuronal promoter [5]. A mouse model with a transgene expressed in the pancreas has also been described (NORBA mice) [6]. Here an AD mouse model was used overexpressing human APP in the brain of heterozygotic transgenic mice, with mutations K670N, M671L, the Swedish variant ($HuAPP_{695}SWE$) [7]. The transgenic expression of APP in the brain leads to increased concentrations of Aβ_{42} and to the formation of amyloid plaques during aging with disease symptoms detectable as behavioral changes between 12–18 months of age. At early stages diffuse amyloid deposits can be detected in brain sections by immunohistochemical analysis or by enzyme-linked immunosorbent assay (ELISA) in brain extracts [7], at later stages plaques become detectable by dyes such as thioflavins or Congo red [3].

Several studies with APP-transgenic mice have demonstrated that active or passive immunization against the Aβ peptide leads to a reduction in amyloid plaque burden and an improvement in memory impairment [8, 9]. Monoclonal antibodies generated previously were directed against the N-terminal 12–16 residues of the peptide and cross-react with the monomeric peptide [10, 11], possibly due to the fact that amyloid aggregates exhibit an exposed unstructured N-terminal region [12]. Conformational antibodies against the Aβ fibrils have also been reported [13]. Another monoclonal antibody, m266, directed against a central region of Aβ (amino acids 13–28) reduced the amyloid burden of the brain in APP-transgenic mice and reversed memory defects [14, 15, 16]. After peripheral injection of m266 plasma levels of Aβ peptide increased rapidly, presumably by facilitating the efflux of soluble Aβ peptide from the brain. Two other antibodies, AMY-33 and 6F/3D, were raised against Aβ peptides 1–28 and 8–17, respectively, and prevented aggregation in vitro [17]. One antibody is known, 3D6, which binds to the amino terminal amino acids and rapidly induced a reduction in amyloid plaque burden in vivo [18]. Here two commercially available antibodies 4G8 [19] and 6H1 were used as controls. They are directed against the amino acid residues 17–24 and 1–17 of human and mouse Aβ, respectively.

In a previous study antibodies against Aβ plaques were produced by using aggregated Aβ_{42} peptides in conjunction with Freund's complete adjuvant at a dose of 100 µg antigen per injection for a total number of 11 immunizations [20]. Five different peptide immunization procedures have been recently compared [21]. A liposome-based vaccination has also been described, which caused a reduction in Aβ plaques in the pancreas of transgenic mice [6]. Peptide immunizations in patients caused inflammation in the brain and led to the halt of a clinical trial [22].

Vaccination with plasmid DNA encoding tumor-associated antigens or antigens derived from various pathogens leads to the expression and presentation of foreign antigens over a prolonged period of time at low levels, inducing both broad humoral and cellular immune responses (reviewed in [23, 24, 25]). These antigen-specific immune responses can be further enhanced by boosting with corresponding peptides, proteins or viral vectors expressing the antigens [24, 26]. DNA vaccination induces Th1 or Th2 type immune response depending on the route of injection, amount of DNA, and the nature of the antigen [27].

In this study we describe an effective vaccination procedure using plasmid DNA coding for Aβ_{42} in combination with a low dose of the aggregated Aβ_{42} peptide in the absence of adjuvants. The rationale for this combined vaccination approach is to provide an aggregated Aβ_{42} peptide nucleus for the plasmid DNA encoded Aβ_{42} peptide and to boost the primary immune response raised by the ectopically expressed peptide. This immunization leads to a rapid generation of Aβ_{42} peptide specific antibody titers detectable after dilution of up to 1:1.000 within 2 weeks, which can be increased by further inoculations. One antibody recognized the aminoterminal sequence DAEF and differs from other known antibodies. This mouse was used for preparation of monoclonal antibodies. Using sera and monoclonal antibodies for passive immunization we demonstrate solubilization of preformed aggregates of Aβ_{42} in vitro, staining of plaques in brain sections, and lysis of plaques in vivo of transgenic APP mice. Thus we describe a novel type of DNA peptide vaccine for active immunization which may be better tolerated when applied to patients than the peptide only vaccine, which recently failed in patients. Furthermore we describe a passive immunization which may lead to new insights on disease mechanism and a therapy of AD.

Material and methods

Plasmids

For the construction of pVR1012tPA-Aβ_{42}, the coding sequence of Aβ_{42} was amplified by PCR and cloned into the expression vector pVR1012-tPA [28] using the restriction sites *Nhe*I and *Bam*I. The construction of pVR1012-muIL-12 and pVR1012 granulocyte-macrophage colony-stimulating factor (GM-CSF) have been described earlier [29]. Large-scale preparation of plasmid DNA was performed as previously described [29].

Peptides

For immunization, Aβ_{42} peptide [1] (Bachem, Bubendorf, Switzerland) was dissolved in PBS and aggregated by incubation on a shaker for 48 h at 37°C at a concentration of 10 mg/ml. For intramuscular injection, aggregated Aβ_{42} peptide was suspended in PBS to a concentration of 2 mg/ml.

Cell cultures

COS7 cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 2 mM glutamic acid, and penicillin/streptomycin. Cells were transfected with pVR1012 control vector or pVR1012tPA-Aβ_{42} using the calcium phosphate method [30]. At 72 h posttransfection the cells were subjected to immunofluorescence analysis using the monoclonal antibody 6H1 (Evotec Neurosciences, Hamburg, Germany) for detection of human Aβ.

Active immunization

For active immunization wild-type mice from SWE breeding were used at 4–6 weeks of age. DNA (200 µg in 200 µl PBS) was injected intramuscularly into both hind legs. Where indicated, Aβ_{42} encoding DNA was injected in combination with 100 µg DNA encoding either interleukin (IL) 12 or GM-CSF (total volume 200 µl PBS). For peptide immunization mice were injected into both hind legs with 20 µg Aβ_{42} peptide in 200 µl PBS without adjuvant. All mice were immunized at 4-week intervals for up to 12 immunizations. Blood samples were taken 2 days after every vaccination. For control purposes vaccinations were performed with DNA or peptide only.

Passive immunization

Tg(huAPP$_{695}$.SWE; SwAPP) mice at 16–20 months of age were passively immunized with 200–300 µl undiluted sera collected from mice that had been actively immunized as indicated. SwAPP mice were injected four times every 3–4 days, killed 24 h after the last immunization, and analyzed by immunohistochemical staining or ELISA [7]. SwAPP mice receiving sera from mice immunized with DNA peptide combinations were treated once for 1 day or twice at 2-days intervals and killed 1 day later.

Quantification of Aβ_{42} in brain lysates

For quantification of sodium dodecyl sulfate (SDS) soluble Aβ brain tissue was homogenized in lysis buffer containing 2% SDS, 1% Triton X-100, 20 mM Tris (pH 7.6), 137 mM NaCl, and 1× complete proteinase inhibitor mixture (Roche Molecular Biochemicals, Basel, Switzerland) [7]. Cleared lysates were collected following centrifugation at 25,000 *g* for 30 min at 4°C. The concentration of Aβ_{42} peptide in each sample was determined by ELISA (Innogenics, Switzerland) according to the provider's instructions. Statistical significance of the antibody titers raised against the Aβ_{42} peptide was determined by Student's *t* test.

Aβ_{42} ELISA for serum antibodies

Antibodies specific for the Aβ_{42} peptide were detected and quantified by ELISA using disaggregated Aβ_{42} peptide as described [31].

Thioflavin T binding assay

The disaggregation capacity of mouse sera and monoclonal antibodies in vitro was determined by the amount of thioflavin T binding to β-sheets using the thioflavin T-binding assay or ELISA as described [6, 12]. Statistical significance of the solubilization capacity of individual antibody clones was determined by Student's *t* test.

Generation of Aβ_{42}-specific monoclonal antibodes

Spleen cells of mouse 697 were fused with myeloma NS-1 cells for generation of monoclonal antibodies as described [32]. Hybridoma cells producing antibodies specific for Aβ_{42} peptide were identified by Aβ_{42} ELISA. Five clones were subjected to subcloning and grown to mass cultures. The antibodies were purified by affinity chromatography before immunohistochemical use.

Epitope mapping

For epitope mapping 13mer peptides with two amino acid overlaps spanning the Aβ_{42} sequence were synthezised using SPOT synthesis and immobilized on filters or glass chips using 0.3–0.6 mol per spot at Jerini AG, Berlin [33]. The peptide supports were incubated with sera at 1:1.000 dilution as described [34]. Peptide bound antibodies were detected using a peroxidase-labeled anti-mouse IgG polyclonal antibody together with a chemiluminescent substrate and the LumiImager (Roche Diagnostics, Mannheim, Germany).

Immunohistochemistry

Sera from actively immunized wild-type mice were diluted 1:10, 1:100, and 1:1.000 in PBS and used for detection of amyloid plaques in paraffin embedded brain sections of a 12 months old transgenic SwAPP mouse. The monoclonal antibody 4G8 (1:1.000 diluted; Signet Pathology Systems, Dedham, Mass., USA) was used as the positive control. To determine the number of amyloid plaques of passively immunized mice, coronal brain sections (5 µm) were immunostained with the antibody 4G8 (dilution 1:1.000) and plaque burden was estimated in five individual sections of each animal.

Results

Cloning and expression of Aβ_{42}

The plasmid comprises the human cDNA coding for the Aβ_{42} fused to the tissue-type plasminogen activator (tPA) secretory signal under the control of the cytomegalovirus – immediate early enhancer/promoter region and the pVR1012 backbone (Fig. 1a). Expression of Aβ_{42} was verified by immunofluorescence staining with the antibody 6H1 in transiently transfected COS7 cells (Fig. 1b)

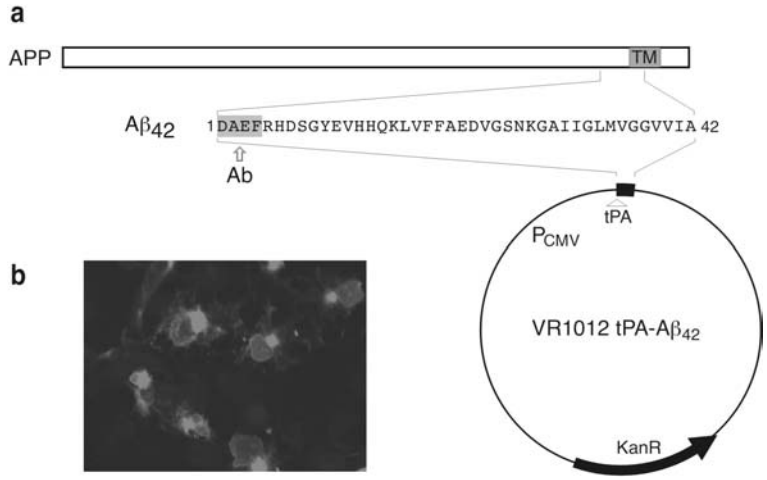

Fig. 1 a The plasmid DNA coding for Aβ_{42} contains the secretory signal sequence of tissue-type plasminogen activator (tPA), the cytomegalovirus (CMV) promoter P_{CMV}, and the Kanamycin resistance gene KanR. VR1012 indicates the plasmid backbone. **b** COS7 cells were transfected with the plasmid pVR1012tPA-Aβ_{42} and analyzed for Aβ_{42} protein expression by immunofluorescence analysis using the antibody 6H1. Of Aβ_{42} peptide 24 pg is expressed in 10^5 cells and 1.9 pg in the supernatant of 10^5 cells

and quantified by ELISA for the expression of human Aβ_{42}. The amount of Aβ_{42} secreted into the culture supernatant was 1.9 pg per 10^5 cells in 48 h. The cell lysate contained 24 pg Aβ_{42} per 10^5 cells.

Immunization and antibody titers

For active immunization DNA coding for Aβ_{42} was used in combination with aggregated Aβ_{42}. Immunizations were performed by intramuscular injection of DNA (200 μg) and peptide (20 μg) or as indicated in the individual experiments. No adjuvant was used. This is in contrast to previous peptide vaccinations, where much higher amounts of peptide were administered combined with Freund's complete adjuvant, phage coupled to bovine serum albumin or liposomes [6, 10, 20]. Antibody titers determined by ELISA showed that the combination of DNA and peptide vaccination described here resulted in detectable antibody titers at a dilution of 1:1.000 after only one immunization (Fig. 2a). The observed increase in antibody titers specific for Aβ_{42} was significant 6 (P<0.098), 8 (P<0.02) and 10 weeks (P<0.00001) after the initial injection compared to the control vaccination with empty backbone DNA (Fig. 2a). The DNA peptide vaccination induced higher titers up to 1:10.000 after four to eight injections (P<0.016 and P<0.088, respectively, compared to vaccination with empty vector DNA), which further increased with more immunizations (Fig. 2b). Combination of Aβ_{42}-encoding DNA with DNA encoding IL-12 (data not shown) or GM-CSF without peptide induced very low antibody titers (Fig. 2b). Control experiments showed that the peptide alone gave a weak and transient response. Empty backbone DNA control and DNA encoding Aβ_{42} alone were both negative. The combination of backbone DNA and peptide, however, gave rise to significant Aβ_{42}-specific antibody titers, 8 and 10 weeks after the first vaccination (P<0.0279, and P<0.0043, respectively). This may be attributable to CpG sequences in the DNA backbone (Fig. 2a, open circles) [35].

In vitro lysis of aggregates by thioflavin binding assay

To determine the ability of antibodies to dissolve Aβ peptide aggregates in vitro we aggregated Aβ_{42} by incubation at 37°C for 7 days without agitation [3]. This aggregate binds the fluorescent dye thioflavin T and allows determination of the degree of solubilization, measured either by ELISA of released solubilized Aβ peptides or as a shift of absorbance and emission from 380 and 430 nm to 450 and 480 nm of thioflavin bound to Aβ aggregates, respectively. Figure 3a shows one of three independent experiments in which mouse sera were tested by ELISA whereby mouse 697 exhibited the strongest disaggregation capacity in vitro. It was vaccinated with a combination of Aβ-encoding DNA and peptide. Monoclonal antibodies were generated from this mouse, concentrated 50-fold from hybridoma supernatants and tested for their disaggregation capacity using the thioflavin binding assay (Fig. 3b). The clones 16E6H9 (P<0.0024), 14E6C9, (P<0.015), and 16G1F12, (P<0.015) showing significant solubilization of aggregated Aβ_{42} were selected for further subcloning.

Epitope mapping of antisera

The sera obtained were analyzed for their ability to bind to linear peptides derived from Aβ_{42}. Sixteen consecutive peptides 13 amino acids in length with two amino acids overlaps were spotted onto filter paper. Binding was quantified by chemiluminescence (Fig. 4) [33]. Serum

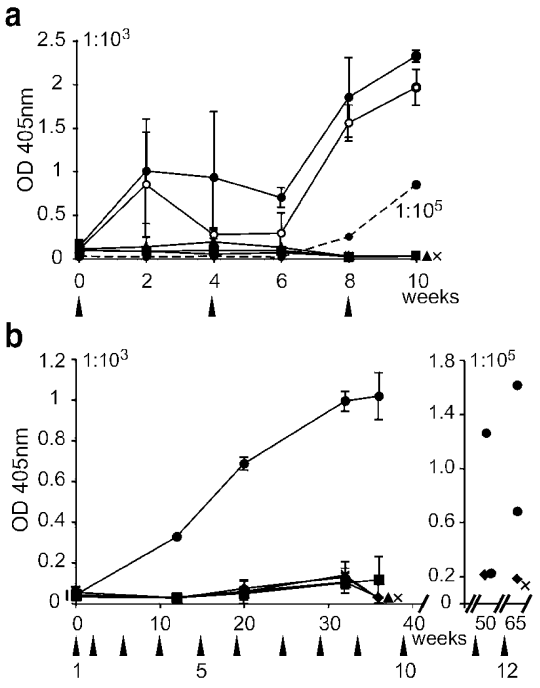

Fig. 2 Antibody titers. **a** Mice were immunized with plasmid DNA encoding Aβ_{42} and the analogous peptide at the indicated time points (*arrows*). The vaccination was performed with three to four mice per group. The titers were determined by ELISA with monomeric Aβ peptide. *Filled circles* Aβ encoding DNA and Aβ peptide combined; *open circles* pVR1012 backbone DNA and Aβ peptide; *X* pVR1012 backbone DNA alone; *filled triangles* Aβ encoding DNA alone; *filled squares* Aβ peptide alone (all three baselines); *dotted line* is from the serum of a mouse immunized with Aβ-DNA peptide combination diluted $1:10^5$, all others from sera diluted $1:10^3$. **b** Vaccination was performed in two independent experiments using three mice per group with injections as indicated. Results were combined. Dilution was $1:10^3$. *Filled circles* Aβ encoding DNA and Aβ peptide combined; *X* pVR1012 backbone DNA; *filled triangles* Aβ encoding DNA alone; *filled squares* Aβ peptide alone (all three baselines); *filled diamonds* Aβ encoding DNA and GM-CSF encoding DNA combined. Some individual mice that received 11 or 12 immunizations are shown with titers $1:10^5$ (*right*)

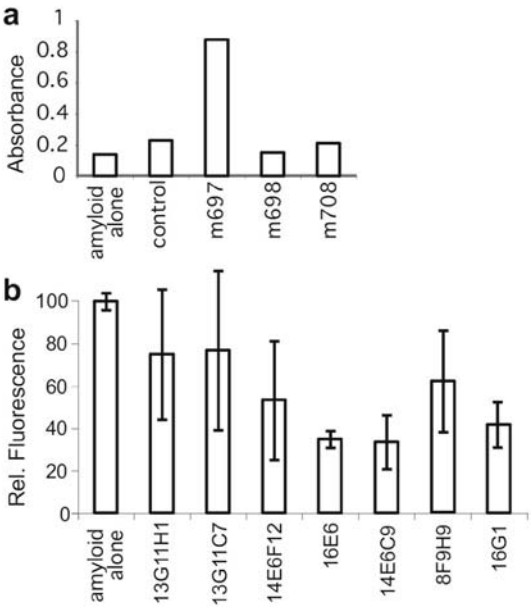

Fig. 3 Disaggregation tested by ELISA for solubilized Aβ complexed with antibodies. Aβ peptide was aggregated on microtiter plates (7 days) and treated with antibodies. The solubilized Aβ as immune complex was determined by ELISA measuring the absorbance at 405 nm (**a**). Alternatively, 50-fold concentrated hybridoma supernatant was added and the amount of thioflavin bound to β-sheets in the lysate measured by fluorescence. Seven of 16 clones are shown. *Error bars* Standard deviation of three measurements (**b**)

from mouse 697 preferentially bound to the very N-terminal region of the Aβ peptide comprising the amino acid sequence DAEF. A second serum from mouse 702, which received DNA encoding Aβ only, showed a much reduced binding by about tenfold with no preference for any sequence. Antibodies obtained from mice 707 or 709, which received DNA encoding Aβ plus DNA encoding GM-CSF or IL-12, showed low binding to all or to central epitopes, respectively.

Reactivity of sera with amyloid plaques

Frontal brain sections from a 12 months old mouse transgenic for human APP (tgHuAPP$_{695}$SWE) [5] were incubated with antisera at dilutions from 1:10 to 1:1.000 obtained from actively immunized mice and were compared to a commercially available control antibody 4G8. Six sera were tested using five slices for each (Fig. 5a). The results show that the serum of mouse 697, which was immunized with the Aβ-encoding DNA and peptide combination, stained the plaques at a dilution of 1:100 (Fig. 5a, C). Mice immunized with DNA encoding Aβ_{42} and DNA encoding GM-CSF (mouse 707) or IL-12 (mouse 709) showed staining of neurons only (Fig. 5a, F) or no staining at all (Fig. 5a, G), respectively. Sera of all other mice were negative for staining. Antiserum from mouse 3583, which was immunized by peptide and control vector, gave rise to staining of plaques (Fig. 5a, H). Two monoclonal antibodies derived from mouse 697, 16G1, and 16E6, harvested from hybridoma supernatants, both containing 42.5 mg/ml, allowed staining of plaques at a dilution of 1:1000, whereby 16E6 was specific for plaques and 16G1 also recognized APP (Fig. 5a, I, K).

Plaque reduction in vivo by passive immunization

For passive immunization transgenic mice at the age of 16–20 months received antisera from mice vaccinated with the Aβ-DNA peptide combination, DNA encoding Aβ_{42} alone, DNA encoding Aβ_{42} combined with DNA encoding IL-12, GM-CSF, or only empty backbone as control DNA. A quantity of 250–300 µl of the sera was injected into the tail veins. Serum of mouse 697, vaccinated with the Aβ-DNA peptide combination, was injected once (300 µl) in one mouse, which was killed 1 day

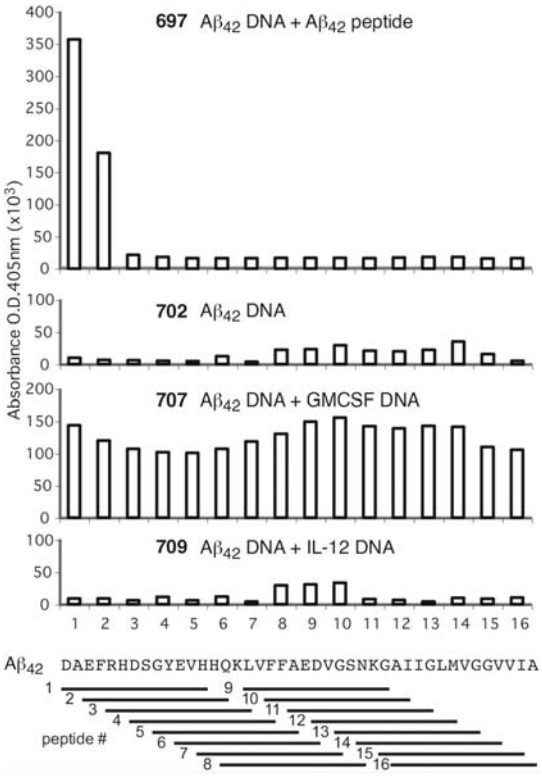

Fig. 4 Peptide scanning. Sixteen consecutive peptides, 13 amino acids in length, which overlapped by 11 amino acids with both neighboring peptides, were synthesized, spotted onto membranes, and analyzed with the sera indicated for recognition of epitopes. Mouse 697, which recognized the aminoterminal sequence DAEF, was used for preparation of monoclonal antibodies

later. Another mouse was injected twice within 3 days and killed 1 day later. Six brain sections of each immunized mouse were taken throughout the cortex and hippocampus and stained with the control mAb 4G8. The number of plaques was evaluated by microscopy and compared to those of untreated mice. As can be seen in Fig. 5b, the amyloid plaque burden was low 24 h after a single injection (Fig. 5b, C). With two injections within 4 days no plaques were detected (Fig. 5b, D). Sera from the other mice showed no reduction in plaques (Fig. 5b, E–H).

As an alternative for analyzing brain sections of aged transgenic mice younger mice at about 5 months of age were passively vaccinated and the levels of Aβ_{42} in SDS extracts of brains were determined by ELISA [7]. Passive immunization with two of the monoclonal antibodies (clones 16E6 lanes 5–8 and 16G1 lanes 9–13) showed a significant reduction in brain levels of Aβ_{42} (Fig. 5c). Lanes 1–4 show untreated control (lanes 3 and 5 failed).

In addition to the evaluation of plaque reduction in the brains of human APP transgenic (tgHuAPP$_{695}$SWE) mice by passive immunization, we also wished to test the combined DNA vaccine Aβ_{42} peptide active immunization protocol. However, the overexpression of the human APP protein in transgenic (HuAPP$_{695}$.SWE) mice leads to an increased spontaneous mortality in an age-dependent manner. Under standard laboratory conditions only approx. 50% of the animals reach an age of 1 year. Unfortunately, too many of the heterocygotic APP transgenic mice died during the study to allow a meaningful interpretation of the results. However, the observed mortality rate of mice actively immunized with DNA, peptide, or a combination of the two was not increased when compared to untreated control animals (data not shown).

Discussion

Vaccination against the amyloid Aβ protein is of high interest. Here we describe a vaccination procedure using plasmid DNA combined with peptides. A few injections were sufficient to raise relevant antibody titers in all eight mice. The titers increased with the number of inoculations. DNA as a vaccine has been applied in many preclinical as well as clinical settings and proved to be extremely safe but of rather low efficiency [36]. The combination of DNA with peptide boosts increased the efficiency, by which both the humoral and the cellular immune response were involved. DNA prime peptide boost regimens are about to enter clinical trials as vaccines against various pathogens such as human immunodeficiency virus and Ebola virus. A long-term follow-up study in patients has not shown any signs of anti-DNA antibodies as a risk for autoimmune diseases and was well tolerated without any side effects [37].

Peptide vaccines have been tested previously in combination with Freund's adjuvant, liposomes, phages and by different routes [6, 10, 20, 21]. In most previous tests 50–100 µg of peptide per injection was used. Recently patients with progressed AD were treated with amyloid Aβ peptide vaccines in a clinical trial. It resulted in the occurrence of meningoencephalitis in some of the patients and led to a halt of the clinical trial [22, 31]. The DNA construct combined with the aggregated Aβ peptide used here induced antibodies that specifically recognized the very aminoterminal four amino acids DAEF of the amyloid Aβ peptide. The serum when injected intravenously for passive immunization of SwAPP transgenic mice 16–20 months old induced a rapid reduction in plaques within 24 h after treatment.

Passive immunizations have been described in transgenic mice with m266 (recognizing N-terminal sequences) [14, 15, 16] and monoclonal antibodies 3D6 and 10D5 (recognizing N-terminal sequences) [18]. They bind to Aβ plaques and some lead to reduction of plaque burden (for review see [38]). m266 reversed memory defects in learning and recognition tasks [16]. The monoclonal antibodies described here were derived from the mouse 697, which produced serum against DAEF, and differ from sera previously described. Reduction in the amount of Aβ in the brains of young (5 months of age) and amyloid plaques of old (16–20 months of age) AD mice was observed here. The reduction was very rapid and occurred within 24 h after a single injection of 200–300 µl undiluted sera. A rapid biological response has previously been reported and was attributed to Fc-medi-

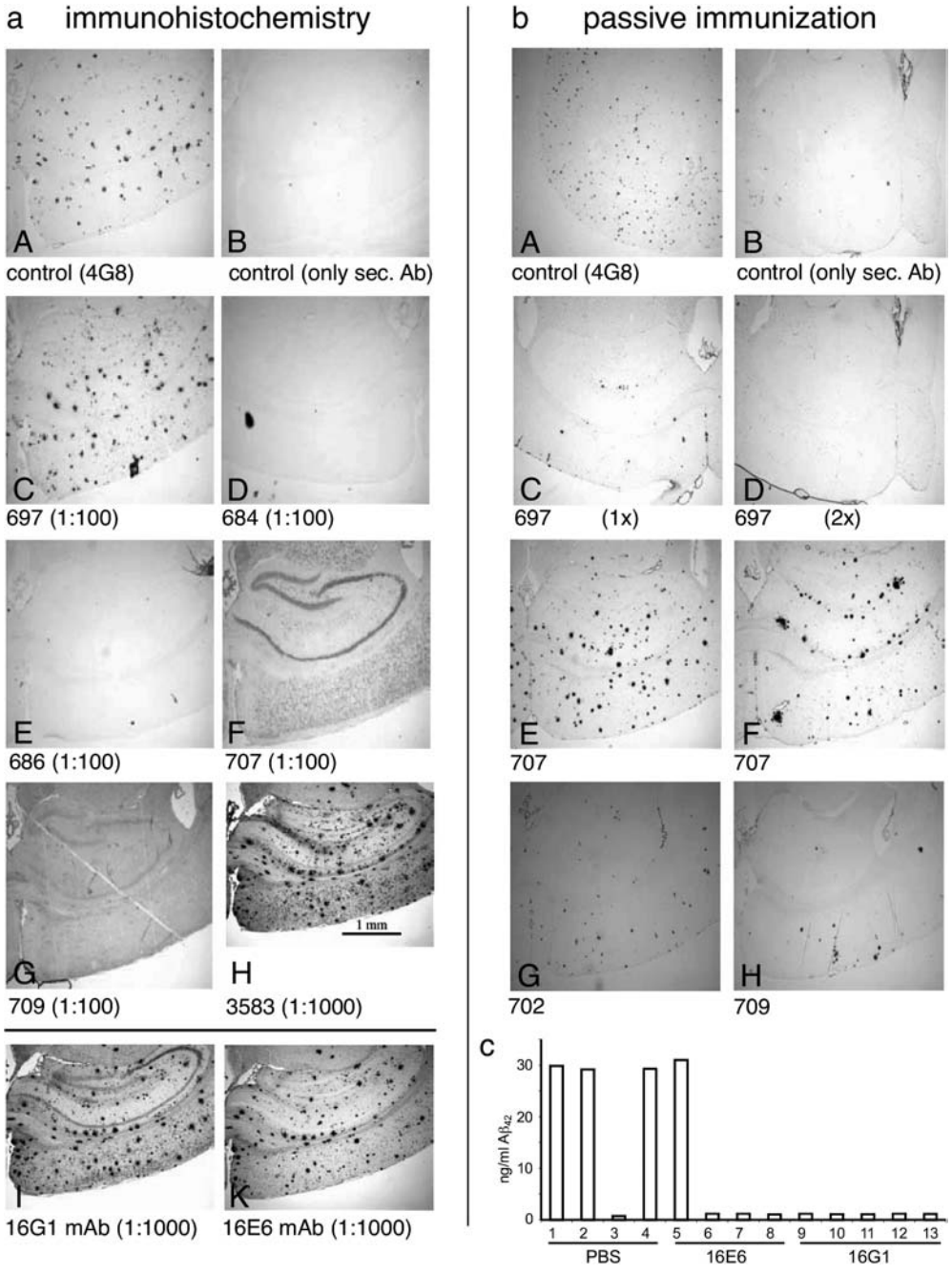

Fig. 5 a Immunohistochemical staining of amyloid plaques in brains of 12 months old transgenic APP mice by sera of immunized mice. Monoclonal antibody 4G8 served as positive (*A*), the secondary antibody as negative control (*B*). Sera were tested at the dilutions indicated. Serum of mouse 697 immunized with Aβ DNA and peptide (*C*), mice 684/686 empty vector control (*D*, E), mouse 707 Aβ DNA and GM-CSF DNA (*F*), mouse 709 Aβ DNA and IL-12 DNA (*G*), mouse 3583 backbone DNA and peptide (*H*). Purified monoclonal antibodies (mAb;)diluted to the final concentration of 42.5 µg/ml) derived from mouse 697 are 16G1 (*I*) and 16E6 (*K*). **b** Passive immunization of 16–20 months old transgenic Aβ mice. Sera from mice immunized with various combinations of DNA and/or peptide were injected into the tail vein of transgenic mice. The mice were killed, and the brain sections were stained with 4G8 positive control antibody (diluted 1:1.000) (*A*). The secondary antibody control is shown in *B*. For passive immunization serum obtained from mouse 697, vaccinated with Aβ DNA and peptide, was injected once (*C*) or twice at 2-day intervals (*D*) and analyzed 1 day later by mAb 4G8. Six slices of each brain were tested with similar results; only one is shown. Other sera for passive immunizations were from mice immunized six to eight times with a combination of Aβ DNA and GM-CSF DNA (serum of mouse 707, *E*, *F*), Aβ DNA alone (serum of mouse 702, *G*), Aβ DNA and IL-12 DNA (serum of mouse 709, *H*). **c** $A\beta_{42}$ Contents in brain lysates of passively immunized mice. Transgenic mice 5–6 months old were treated intravenously with purified antibodies from hybridoma supernatants 4 times at 4-day intervals (250 µg in 200 µl per injection). The mice were then killed and Aβ aggregates analyzed from brain homogenates by ELISA. Mice 1–4 received buffer, mice 5–8 were passively immunized with the monoclonal antibody 16E6, and mice 9–13 with 16G1. Mice 3 and 5 were drop-outs

ated phagocytosis of microglial cells [18]. The alternative theory of a "peripheral sink" has also been proposed, whereby the formation of antigen-antibody complexes in the periphery sequesters Aβ peptides and prevents the deposition of new plaques. Alternatively, plaques may be physically disrupted by the antibody. The short time period required for disruption of plaques may be due to physical or conformational changes in the plaques induced by the antibody. A monoclonal antibody of this kind has been described for the scrapie prion PrP^{sc} by Prusiner and colleages [39]. However, passive immunization has recently been performed in APP23 transgenic mice with 20 weekly injections of 500 µg monoclonal antibody that recognizes amino acids 3–6. After 5 months the mice developed cerebral hemorrhages, presumably due to the weakening of amyloidotic vessel walls by local inflammatory reactions [40].

The effect of empty DNA plasmid controls combined with the Aβ peptide was unexpected since the antibody titer and specificity resembles that with Aβ DNA. It may be possible to use only CpG oligonucleotides with the Aβ peptide for clinical use.

In summary, we present two new aspects, a new active immunization procedure with a Aβ-DNA peptide combination and a new monoclonal antibody for passive immunization. The combined DNA peptide vaccine differs significantly from all previous ones since no adjuvant was used and the amount of peptide used, 20 µg, per injection was very low. This is important in view of the suspicion that the use of adjuvants may support a Th1-mediated immune response, which has been implicated in autoimmune diseases and may have contributed to the adverse response in some patients vaccinated with the Aβ_{42} peptide. Moreover, analysis of the Ig subtype of the Aβ_{42} peptide-specific monoclonal antibodies, revealed that all of them were of the IgG1 subtype. This finding indicates that the combined DNA vaccine/Aβ_{42} peptide vaccination approach induces a Th2 type of response, which may be an important advantage by inhibiting an autoimmune reaction in patients. A few injections of the DNA peptide combination gave rise to significant antibody titers which could be increased by additional boosts. Furthermore, a monoclonal antibody may be useful for characterization of molecular mechanisms underlying the rapid solubilization of plaques in vivo and in vitro and may be developed into a therapeutic vaccine applied at early stages of AD to prevent disease progression.

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