УДК 617:616-073.1

Comparative analysis of anti-amyloid β immune responses generated after different routes of immunization with plasmid encoding Aβ-peptide immunogen fused with IL4

D.T. Babikyan

The Institute for Molecular Medicine
16371 Gothard Str., Huntington Beach, CA 92647

The Center of Medical Genetics of NAS of Armenia
Zakyan 5/1, Yerevan 375010, Armenia

Key Words: Alzheimer's disease, Aβ peptide, immunization, DNA vaccine

Alzheimer's Disease (AD) is the most common form of dementia that increases in incidence with age. The pathology of the disease is associated with the presence of neuritic and diffuse plaques in the brain. The main components of plaques, $A\beta_{42}$ or $A\beta_{40}$, are 42 or 40 amino acid peptides that result from abnormal cleavage of membrane bound Amyloid Precursor Protein (APP) by the β and γ secretases. Recently several groups have reported that immunization with Aβ peptide formulated in CFA/IFA adjuvant dramatically increases the clearance of amyloid plaques from brain tissues in APP/Tg mice models of AD. In addition, active immunization not only protects vaccinated mice from functional memory deficits but also improves pathology of AD-like disease in older animals [5,15,19,27]. On the other hand passive administration of anti-Aβ monoclonal antibodies to APP transgenic mice also resulted in the decrease of Aβ level in the brain and improved the pathology of AD-like disease [3, 8]. More recently, it has been demonstrated [9] that passive immunization in fact could rapidly reverse memory deficits in an object recognition task in 24-month old PDAPP (APP gene driven by a platelet-derived (PD) growth factor promoter) mice without altering brain Aβ burden. Thus, the results reported by different groups indicate that Aβ is a major target for a vaccine development against AD.

Based on these impressive results and the lack of adverse autoimmune-type reactions to Aβ-immunotherapy in the several animal models (mice, rabbits, guinea pigs and monkeys), Elan in collaboration with Wyeth-Ayerst began clinical trials with their AN-1792 vaccine on AD patients. Unfortunately, the trial was halted when approximately 5% of the participants developed some degree of meningoencephalitis [4,12, 28,29,33]. The cause of the meningoencephalitis in a subset of the patients has not yet been determined, and all of the patients were reported to respond favorably to treatment for the meningoencephalitis [28]. Interestingly, the antibody titers did not correlate with the severity of signs and symptoms in the AN-1792 clinical trial, and some of the patients that developed meningoencephalitis did not have detectable levels of anti-Aβ antibodies [13]. This suggests that the adverse reaction to Aβ-immunotherapy was not due to the humoral antibody response, but rather to a T helper 1 (Th1) cell-mediated autoimmune response to AN-1792. This interpretation of the adverse response to AN-1792 is supported by the case report by Nicoll et al. [21] on the neuropathology of human Alzheimer's disease after immunization with multiple doses of the AN-1792 vaccine. Examination of the brain revealed the presence of T lymphocyte meningoencephalitis and infiltration of macrophages in white matter areas. The potentially exciting results from the case report were that extensive areas of the neocortex contained very few Aβ-plaques. This suggests that Aβ-immunotherapy may be capable of reducing the amyloid load in the human brain as was previously shown for APP/Tg mice. Additional data supporting the concept of Aβ-immunotherapy have recently been reported by Hock et al. [14]. These authors provided the first evidence that antibodies against Aβ can slow the cognitive decline in AD patients (n=30) involved in the AN-1792 clinical trial. Although, the study by Hock et al. [14] was small (a total 30 patients) and the meningoencephalitis side effect remained a problem, the data presented showed that the concept of vaccination may be possible [34].

Another strategy is to use passive DNA vaccination technique. Previously using this technique, potent humoral and cellular immune responses were generated against viral and tumor antigens [1,2,10,25,32]. Accordingly, in this study several plasmids were constructed for inoculation of mice by three different routes of immunization.

Materials and Methods

Mice

Eight or ten week-old female BALB/c and B6/SJLF1 mice were purchased from Jackson laboratories and housed in the animal facility at the Institute for Brain Aging and Dementia, UCI. All animals were housed in a temperature and light-cycle controlled facility.

DNA Constructs

In order to develop DNA (genetic) vaccine against AD, four different plasmids were constructed: pmAβ_{42}, encoding transmembrane form of Aβ42; psAβ_{42}, encoding secreted form of Aβ_{42}; pAβ_{28}-IL4 and pAβ_{42}-IL4 encoding chimeric proteins, where marina IL4 is fused with human Aβ_{28} (spanning first 28 were Aβ_{42}) or Aβ_{42} respectively. To generate these constructs, DNA fragment encoding Aβ_{42} and Aβ_{28} peptides was amplified on human APP cDNA template by PCR. The pmAβ_{42} was constructed by cloning Aβ_{42} PCR fragment into pVAC (Invivogen) expression vector in the frame between IL2 signal sequence and C-terminal transmembrane anchoring domain of the placental alkaline phosphatase (PLAP) at BamHI/BglII restriction sites. The psAβ_{42} was generated introducing stop codon at the end of Aβ_{42} sequence and cloning it into pVAC expression vector using BamHI/NheI restriction sites, which allowed to delete PLAP domain from the plasmid. The following primers were used for the construction of transmembrane and secreted forms of Aβ:

5'-GGAAGATCTCTCGCTATGACAACACCGCCCACCATG-3'	reverse for mAβ_{42}
5'-GCTAGCTTACGCTATGACAACACCGCCCACCATG-3'	reverse for sAβ_{42}
5'-GATATCGATGCAGAATTCCGACATGAC-3'	forward for Aβ

In order to use the same pVAC vector for cloning of IL4-Aβ_{42} and IL4-Aβ_{28} fusion proteins, IL2 signal sequence was removed from the vector since IL4 gene contains its own leader sequence. NcoI/BamHI fragment of pVAC vector was amplified by PCR with primers excluding IL2 signal sequence and recloned into the same vector digested with NcoI and BamHI restriction enzymes. Plasmids encoding IL4-Aβ_{42} (pAβ_{42}-IL4) and IL4-Aβ_{28} (pAβ_{28}-IL4) fusion proteins were generated by overlapping PCR technique using the following primers:

5'-GGGCCACCATGGGTCTCAACCCCCAGCTAG	forward for IL4
5'-CCTGAGTCATGTCGGAATTCTGCATCGGCGCCCGAGTAATCCATTTGCATGATGCTCTTTAGG	reverse for IL4
5'-CCTAAAGAGCATCATGCAAATGGATTACTCGGGCGCCGATGCAGAATTCCGACATGACTCAGG	forward forAβ
5'-GCTAGCTACGCTATGACAACACCGCCCACCATG	reverse for Aβ_{42}

Briefly, in the first PCR reaction, two DNA fragments corresponding to murine IL4 or human Aβ peptides (Aβ_{42} or Aβ_{28}) were amplified by the creation of overlapped sequences at expected junctions. In the second PCR reaction the amplified DNA fragments were then combined and the full-length IL4-Aβ_{42} or IL4-Aβ_{28} hybrid DNAs were obtained using forward primer for IL4 gene and reverse primer for Aβ_{42} or Aβ_{28} sequences, respectively. The amplified DNA fragments were treated with BamHI and NheI restriction enzymes and ligated into pVAC vector with deleted IL2 gene leader sequence. The structure of psHA-mC3d3 encoding HA of influenza and 3 copies of C3d component of complement was described previously [26]. The correct sequences of generated DNA constructs were confirmed by nucleotide sequence analysis. All plasmids were purified by Endofree plasmid maxi kit (Qiagen). Purity of DNA was confirmed by UV spectrophotometry (260nm/280nm absorbance ratio >1.7) and gel electrophoresis.

Transfection of cells and expression of plasmids

Transfection of CHO cells with psAβ_{42}, pAβ_{28}-IL4 and pAβ_{42}-IL4 was discussed earlier [10]. To detect the expression of pmAβ_{42} plasmid, $8x10^5$ CHO cells were transiently transfected with 2μg of pmAβ_{42} by Lipofectamine Plus Reagent (Invitrogen) and expression of the plasmid was analysed by immunoprecipitation (IP) in the lysate of transfected cells. Briefly, transfected CHO cells were harvested after 48 h of growth at 37^0C, 10% CO_2 in DMEM-10% FBS by trypsinization, washed with PBS and resuspended in 6M guanidine-HCl. After sonication, the cell lysate was dialyzed against 50mM Tris-HCl buffer containing 0.5M guanidine-HCl. Thc first cell lysate was mixed with 0.5 μg monoclonal anti-Aβ 6E10 antibodies (Signet) and 10μl of Protein G Sepharose beads (Amersham) and incubated at 4^0C overnight. After incubation beads were collected by centrifugation at 1000x *g*, washed twice with PBS, resuspended in electrophoresis sample buffer, boiled 3 *min* and loaded onto 16% Tricine-SDS-polyacrylamide gel. HRP-conjugated protein markers were used as molecular weight standards. Proteins were transferred on the nitrocellulose membrane by electroblotting at constant voltage 100V in 25 mM Tris, 192 mM glycine and 20% methanol as a transfer buffer. Non-specific binding was blocked by incubating membrane in TBS containing 5% nonfat dry milk and 0.05% Tween-20 overnight at 4^0C. The nitrocellulose membrane was incu-

bated with monoclonal anti-human-Aβ 6E10 antibodies (0.2 *mg/ml*) in the same buffer followed by washing with TBS-Tween-20, and incubation with HRP-anti-mouse secondary antibodies and visualized by enhanced chemiluminescence detection using Luminol reagent (Santa Cruz Biotechnology).

Immunization

Immunization of mice with experimental plasmids was performed either by needle intramuscularly (i.m.), intradermally (i.d.) or by gene-gun bombardment into the skin. Groups of eight mice (two independent experiments with 4 animals in each study) were injected i/m (into quadriceps muscle) with 100μg DNA vaccine in 100μl PBS. Before i.d. injection mice were anesthetized with avertin, shaved, and inoculated with 15 μg DNA vaccine in 50μl PBS (1-2 *cm* distal from mouse tail base). Gene gun immunizations were performed on shaved abdominal skin using Helios gene-gun (Bio-Rad) as it was described [26]. Briefly, mice were bombarded twice with doses containing 1 μg of DNA per 0.5*mg* of ~1μm gold beads (DeGussa-Huls Corp.) at a helium pressure setting of 400 psi. Several groups of mice were used as controls. One group of positive control mice (8 animals) were immunized by needle subcutaneously (s.c.) with $A\beta_{42}$ peptide mixed with CFA and boosted biweekly with the same antigen formulated in IFA. Other two groups of positive control mice (8 animals in each) were immunized by needle (i.d. and i.m.) or by gene-gun bombardment with pcHA-3C3d. Finally, a group of eight mice immunized with vector was used as a negative control. Immunization and boosts were carried out by the same method biweekly and sera were collected after 7-8 days of each boost and used for the detection of anti-$A\beta_{42}$ or anti-HA antibodies.

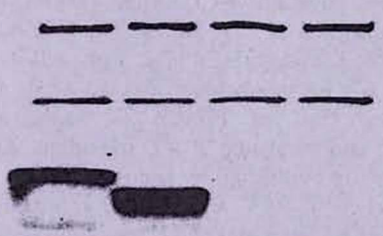


Fig. 1. Expression of $A\beta_{42}$ (lane 1, 2) gene cassettes in CHO cells transfected with appropriate plasmid.
Protein was recovered from cell lysate by IP and analyzed on 16.5% Tricine-polyacrylamide gel followed by WB. In IP and WB monoclonal anti-Aβ antibody 6E10 have been used. Lane 1, lysate of cells transfected with $pmA\beta_{42}$; Lane 2, supernatant of cells transfected $psA\beta_{42}$; Lane 3, supernatant of cells transfected with vector.

ELISA

To detect binding of antisera to immunogen, wells of 96-well plates (Immulon II) were coated with 2.5μM of human $A\beta_{42}$ in Bicarbonate Coating Buffer (pH9.7) and incubated overnight at 4°C. They were then washed and blocked with 3% nonfat dry milk in Tween-20 Tris Buffer Solution (TTBS) (1-2 h at 37°C). After washing of the wells primary sera from experimental and control mice were added in duplicate at indicated dilutions. After incubation (overnight at 4°C) and washing, HRP-conjugated anti-mouse IgG was added as recommended by manufacturer (Jackson Labs). Plates were incubated for 1h at 37°C, washed, and freshly prepared OPD substrate solution (o-phenylendiamine in 0.05M phosphate-citrate buffer, pH 5.0) was added to develop the reaction. All plates were analyzed spectrophotometrically at 405 *nm*. Anti-HA antibodies were detected by ELISA exactly as it was described earlier [26].

Results and Discussion

Preparation and expression of plasmids

The results of transfection and expression of $psA\beta_{42}$, $pA\beta_{28}$-IL4 and $pA\beta_{42}$-IL4 were discussed earlier [10]. Western Blotting analyses revealed that the molecular size of $tmA\beta_{42}$ was slightly higher of 4 *kD* as a result of fusion with anchoring domain of PLAP (Fig. 1). Importantly, transfected cells produced significant quantities of $A\beta_{42}$ since they formed a band, which was much more intensive than the band formed by 5*ng* purified $A\beta_{42}$ peptide. There weren't Aβ peptides in the lysate of the cells transfected with vector DNA.

Generation of antibody responses to Ab peptides

First BALB/c mice were injected i.d or i.m. with $psA\beta_{42}$, $pmA\beta_{42}$, $pA\beta_{42}$-IL4 and $pA\beta_{28}$-IL4. The groups of eight mice were immunized and boosted 3 times biweekly. It appeared that after i.d. immunization and three boosts with plasmid encoding $A\beta_{42}$ peptide alone or $A\beta_{42}$ peptide fused with IL4 did not generate anti-Aβ antibodies. In contrast, positive control BALB/c mice immunized s.c. with $A\beta_{42}$ peptide formulated in CFA/IFA induce robust antibody production as it was expected from the previous data [6]. Importantly, i.d. injection of psHA-mC3d3 (positive control for DNA vaccination) also generated robust anti-HA antibodies after three boosts. Thus, DNA vaccination with control plasmid demonstrated that the technique chosen for this study in general is working very well. Of course mice immunized with vector (negative control group) did not induce either anti-$A\beta_{42}$ or anti-HA antibody production. Of note i.m. immunization of mice with plasmids encoding Aβ immunogen also did not induce production of specific antibodies. Thus, DNA immunization failed to induce humoral anti-Aβ immune responses in case of i.d. or i.m. injections. After failure to induce immune humoral responses by i.m. or i.d. immuni-

zations, it was decided to use more powerful technique – gene-gun bombardment for immunization of mice. Previously it was shown that fusion of gene encoding viral antigen with IL4 gene dramatically enhance humoral immune responses [16]. Thus, in this study it was attempted to use the same strategy to generate anti-AB antibodies in wild-type mice. Again mice immunized with vector did not induce anti-$A\beta_{42}$ antibody production, whereas injection of psHA-mC3d3 generated only anti-HA specific antibodies (data not shown). More importantly, even gene-gun technique did not allow generating anti-$A\beta_{42}$ antibodies after immunization with $psA\beta_{42}$ or pmAb42. In the group of mice immunized with $pA\beta_{42}$-IL4, half of eight experimental mice generated robust anti-$A\beta_{42}$ immune response after only two boosts with $pA\beta_{42}$-IL4, whereas two mice responded moderately, and the remaining two mice responded weakly (Fig. 2a). Titer of antibodies in the pooled sera was detected in two separate experiments (four mice in each experiment) and was equal to 1:3200 (Fig. 2b).

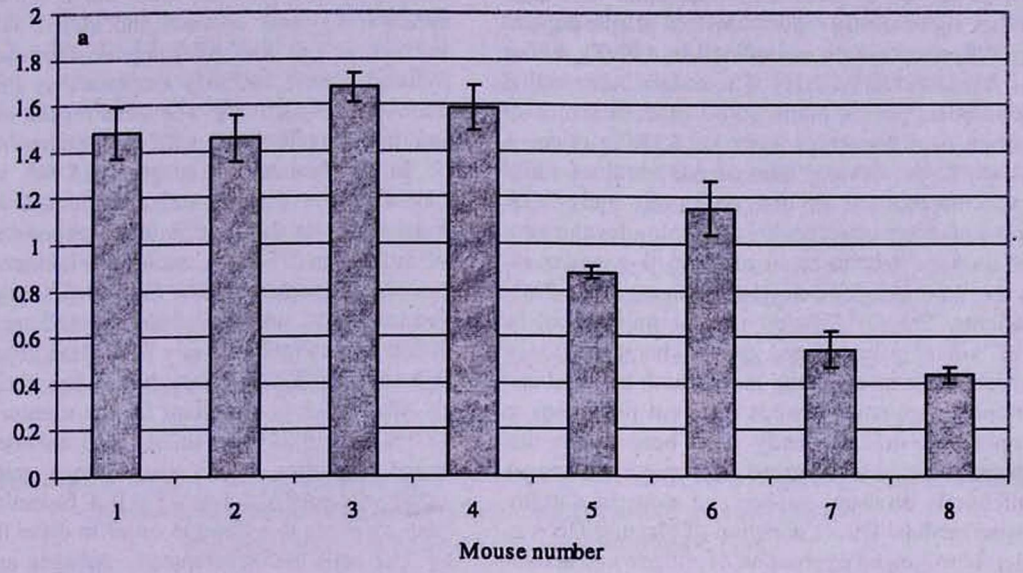
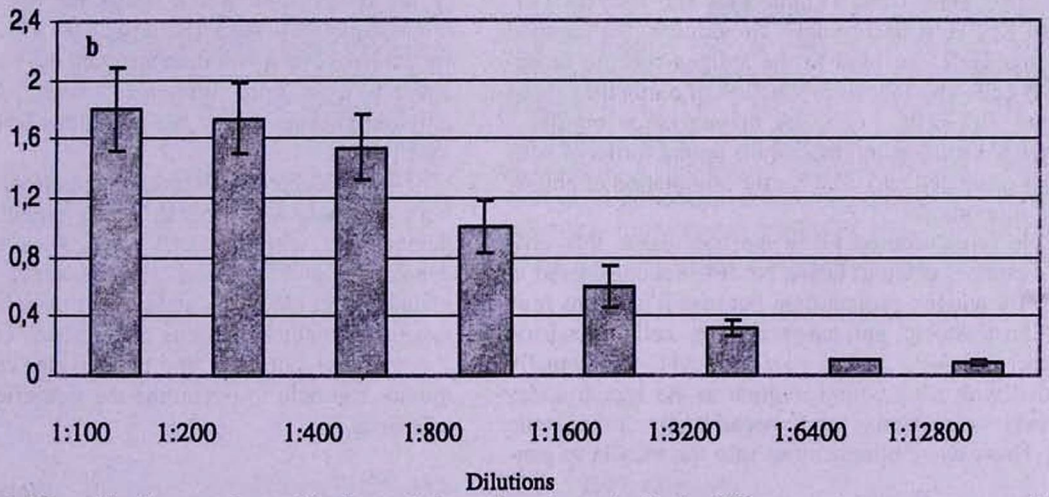


Fig. 2. Generation of anti-$A\beta_{42}$ antibodies after gene-gun immunization of eight B6/SJLF1 mice with plasmids encoding IL4-$A\beta_{42}$ fusion protein. One microgram of plasmid was administrated three times and eight days after the last boost sera were collected and anti-$A\beta_{42}$ antibodies (total Ig) have been detected.
a) Serum of each mouse was diluted 1:250 and used in ELISA (SD represents results from three separate tests with the same sera).



b) Titer of anti-$A\beta_{42}$ antibodies was detected in the pooled sera from immunized mice (SD represent two separate experiments with sera of mice).

Thus, i.d., i.m. and gene-gun immunizations failed to induce significant antibody production in wild-type mice vaccinated with these prototype DNA vaccines, encoding soluble or transmembrane forms of $A\beta_{42}$. But the presence of murine IL4 molecule as a molecular adjuvant in the plasmids encoding human Aβ peptides ($A\beta_{42}$ or $A\beta_{28}$) was critical for the generation of anti-Aβ antibodies after bombardment with gene-gun immunization.

Recently, immunotherapy as a possible treatment for AD has received considerable attention. It has been demonstrated that active immunization of APP/Tg mice with fibrillar $A\beta_{42}$, as well as passive immunization with anti-Aβ antibodies significantly reduce amyloid plaque deposition, neuritic dystrophy, and astrogliosis in APP/Tg mouse models of AD [3,8,15,19,27,33]. These data indicate that anti-Aβ antibodies play a major role in the clearance of Aβ deposition from the brain tissue of APP/Tg mice. A new approach to the development of AD vaccines could be DNA vaccine encoded Aβ antigen. In this study DNA vaccination technique was decided to employ for the generation of immune responses to amyloid β. peptides involved in the deposition of β-amyloid plaques in the brain of AD patients. The DNA-based vaccine belongs to the category of "killed" vaccines and yet has characteristics of a "live" vaccine, because it could induce both humoral and cellular immune responses against different pathogens in different animal models. Recently it has been shown that DNA immunization induces protective immune response against infectious diseases, cancer and even in autoimmune disease models. Direct injection of plasmid DNA in vivo results in prolonged expression of antigen and activation of both humoral and cellular immune responses. Among advantages of DNA vaccination are the versatility in the choice of the route of administration, the relative ease to construct and produce large amounts of recombinant molecules, production of intracellular peptide(s) and secretion of extracellular peptide(s), which can be directed toward MHC class I and II molecules and activate Th1/CTL and Th2 cells accordingly. In addition, the secreted soluble molecule can bind to the antigen-specific receptors on B cells and induce production of antibodies using help from Th2 cells. For DNA immunization constructions encoded soluble and membrane bound forms of $A\beta_{42}$ were first generated and used for the stimulation of anti-β-amyloid antibodies.

Muscle is considered to be the best tissue that efficiently expresses plasmid DNA, but it is not considered to be a site for antigen presentation because it contains few, if any, professional antigen-presenting cells (dendritic cells, macrophages). In this case, few APC that actually get loaded with antigen and migrate to the lymph nodes are poorly stimulatory and occasionally tolerogenic [17,30]. However, immunizations into the muscle in general induce favorable antibody production in DNA vaccinated animals [7,31,32]. In contrast, inoculation of immunogens into the skin tissue leads to the transfection of epithelial cells as well as direct transfection of dendritic cells and thereafter endogenous antigen synthesis and processing. Thus, i.d. immunization results in the production of antibodies and activation of dendritic cells, which migrate to regional lymph nodes and stimulate activation of cellular immune responses [23]. Based on these data, wild-type mice were immunized i.m. or i.d. using $psA\beta_{42}$ and $pmA\beta_{42}$ constructs. Surprisingly, four inoculations of these plasmids into the mice did not induce B cell responses. Control animals inoculated i.d. with psHA-mC3d3, or s.c. with $A\beta_{42}$ peptide formulated in adjuvant induced robust antibody responses to influenza antigen and $A\beta_{42}$, respectively. The same results we obtained after i.m. immunization and gene-gun bombardment.

In addition to advantages of DNA vaccination discussed above DNA vaccination could also manipulate magnitude and nature of immune responses by co-delivery of molecular adjuvants, such as cytokines, co-stimulatory molecules and others. The IL12 and IL4 cytokines help to generate Th1 and Th2 subsets of T cells respectively [20,24] and simultaneously inhibit the generation of opposite subset [11,22]. Thus, to enhance antibody responses to $A\beta_{42}$ which is important for the immunotherapy of AD [3,5,8,9,15,19,27] one should also enhance Th2-type immune responses. Using this strategy, mice were immunized with $pA\beta_{42}$-IL4 or $A\beta_{28}$-IL4. Selection of this dominant cytokine was done in order to drive the development of Th2 cells and subsequently enhance antibody production. As shown in Fig. 2, plasmids encoding both immunogens and murine IL4 induced significant amounts of anti-$A\beta_{42}$ antibodies only in case of gene-gun bombardment. Notably, as shown earlier [10] DNA vaccination with the same chimeric forms of plasmids generated antibodies are mostly of IgG1 isotype, whereas the level of IgG2a is very low which shows the polarization of immune response toward Th2 type. Also these antibodies are functional since it was demonstrated the binding of pooled antisera from mice immunized with $pA\beta_{42}$-IL4 to β-amyloid plaques on the brain sections from a severe AD case [10].

Future studies with immunization of APP/Tg mice with plasmids encoding IL4-$A\beta_{42}$ or/and IL4-$A\beta_{28}$ will demonstrate whether DNA vaccination can reduce the deposition and promote the clearance of β-amyloid plaques from the brain, and protect mice from developing functional memory deficits and whether co-expression of IL4 or other cytokines and chemokines can improve immunity and help to overcome the side effects of standard adjuvants.

Поступила 11.01.04

Հակաամիլոիդ β իմունային պատասխանների ձեւավորման համեմատական վերլուծությունը IL4 գենի հետ միակցված Aβ-պեպտիդ իմունոգենը կոդավորող պլազմիդով տարբեր ճանապարհներով իմունիզացումից հետո

Դ. Թ. Բաբիկյան

Ալցհեյմերի հիվանդության (ԱՀ) բնորոշ առանձնահատկություններից մեկը ամիլոիդ-β պեպտիդի նորմայից ավել ընթացող ճեղքավորումն է, գլխուղեղի նեյրոններում այս պեպտիդի արտաբջջային կուտակումը և նստվածքների ձևավորումը սկավառակների տեսքով: Մի շարք հետազոտական խմբեր ցույց են տվել, որ 15 ամսեկան APP (Amyloid Precursor Protein) տրանսգեն (APP/Tg) մկների ակտիվ ($Aβ_{42}$ պեպտիդի ներմուծումը) կամ պասիվ (մոնոկլոնային հակա-Aβ հակամարմինների ներմուծումը) իմունիզացումը հանգեցնում է գլխուղեղից β-ամիլոիդ սկավառակների արագընթաց մաքրմանը և ԱՀ նմանվող նյարդապաթոլոգիական երևույթների թուլացմանը: Այս տվյալները ցույց են տալիս, որ հակա-Aβ հակամարմինները էական դեր են խաղում ԱՀ բուժման գործընթացում:

Ներկա աշխատանքում փորձ է կատարված ուսումնասիրել հակա-Aβ հակամարմինների խթանումը տարբեր ԴՆԹ պատվաստանյութերով իմունիզացված մկների մոտ: Որպես ԴՆԹ պատվաստանյութեր կիրառվել են պլազմիդներ, որոնք կազմըված են Aβ մինիգեներից ($pmAβ_{42}$ և $psAβ_{42}$) կամ համապատասխան Aβ մինիգենից, որը կցված է մոլեկուլային աղյուսանման՝ մկան IL4 գենի հետ ($pAβ_{42}$-IL4 կամ $pAβ_{28}$-IL4): ԴՆԹ իմունիզացումը և հետագա մի քանի բուստերը (իմունային պատասխանի ուժեղացումը) բերում են հակա-Aβ իմունային պատասխանի ձևավորմանը միայն $pAβ_{42}$-IL4 և $pAβ_{28}$-IL4 պլազմիդներով իմունիզացված մկների մոտ: Այս տվյալները ցույց են տալիս մոլեկուլային աղյուսանման վճռորոշ դերը հակա-Aβ իմունային պատասխանի առաջացման գործընթացում: Բացի այդ, այս աշխատանքում ցույց է տրված նաև, որ միայն գենային «ատրճանակով» պատվաստումն է խթանում պոտենցիալ իմունային պատասխան:

Сравнительный анализ антиамилоидных β-иммунных ответов, образовавшихся после иммунизации разными путями с плазмидой, кодирующей Aβ-пептидный иммуноген, связанный с IL4

Д. Т. Бабикян

Одним из характерных признаков болезни Альцгеймера (БА) является аномальное и внеклеточное накапливание β-амилоидного пептида ($Aβ_{40}$ и $Aβ_{42}$) в виде бляшек на нейронах головного мозга. Различными исследователями показано, что активная (введение $Aβ_{42}$ пептида) или пассивная иммунизация (введение моноклональных анти-Aβ антител) 15-месячных APP трансгенных (APP/Tg) мышей приводит к ускоренному очищению β-амилоидных бляшек из головного мозга и ослаблению нейропатологических признаков, схожих с БА. Эти данные свидетельствуют о существенной роли анти-Aβ антител в лечении БА. Настоящая работа посвящена изучению индукции анти-Aβ антител в мышах, иммунизированных различными ДНК вакцинами. В качестве таковых мы использовали плазмиды, состоящие из Aβ мини-генов ($pmAβ_{42}$ и $psAβ_{42}$) или из определенного Aβ мини-гена, связанного с молекулярным адъювантом – мышиным IL4 геном ($pAβ_{42}$-IL4 или $pAβ_{28}$-IL4). ДНК иммунизация и ряд повторных бустов привели к образованию анти-Aβ иммунного ответа только после вакцинации с помощью $pAβ_{42}$-IL4 и $pAβ_{28}$-IL4 плазмид. Эти данные свидетельствуют о решающей роли молекулярного адъюванта в процессе генерации анти-Aβ иммунного ответа. В дополнение было показано, что только вакцинация с помощью генетического "ружья" позволила индуцировать потенциальный иммунный ответ.

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