Aβ1-42 antibody producing plasma cells in DNA Aβ42 trimer immunized mice reside predominantly in the bone marrow

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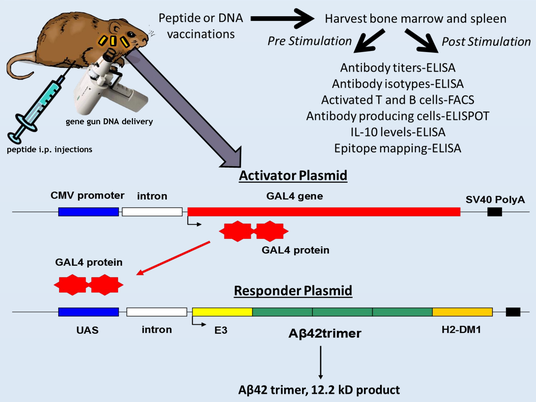
♦ Abstract

Alzheimer's disease (AD) is the most common form of age-related dementia. Immunotherapy provides a possible avenue for prophylaxis of AD, but a clinical trial (AN1792) in which patients with early AD were immunized with Aβ1-42 peptide was halted after the occurrence of meningoencephalitis in 6% of the immunized people, attributed to a T cell autoimmune response. DNA vaccination has been shown to have a polarized Th2 immune response that lacks the inflammatory profile seen in peptide immunizations. In this study, we show a new feature of the DNA Aβ42 trimer elicited B cell immune response and present data for the presence of a long lived plasma cell pool residing within the bone marrow (BM) in DNA immunized mice but not in peptide immunized mice. Two groups of mice were analyzed: one group of B6C3F1 mice (n=20) were studied 4 months after the last DNA vaccination, and a second group of Balb/c mice (n=14), which received DNA or peptide immunizations, were analyzed 10 days after the last immunization. The comparison of antibody producing cells in BM and spleen for the DNA and peptide immunized mice with an ELISPOT assay and subsequent ELISAs showed that BM plasma cells from DNA immunized mice produced more anti-Aβ42 IgG producing cells and higher levels of secreted IgG antibodies. In peptide immunized mice, more IgG antibody producing cells were found to reside in the spleen. These data indicate that the BM may be an important reservoir for B cells following DNA Aβ42 immunization and is in line with studies showing that the BM represents an excellent niche for the survival of long lived plasma cells and a lifetime source for antibody producing B cells which are independent of continuous antigen specific stimulation. Additional studies are needed to further define the phenotype of the antigen specific B cell immune response in DNA Aβ42 trimer immunized mice or differences in the T_H subsets directly involved in initial signaling events to B cells in the germinal center reactions.

♦ Introduction

- Alzheimer's disease (AD) is the most common form of age related dementia
- Deposition of Aβ in the brain is a hallmark for clinical manifestation of AD
- Immunotherapy provides a possible avenue for prophylactic treatment Alzheimer's disease
- A clinical trial (AN1792) in which patients with AD were immunized with Aβ42 peptide was halted because of the incidence of meningoencephalitis in 6% of the immunized group due to a pro-inflammatory immune response
- Passive immunizations with monoclonal antibodies against Aβ42 have been largely ineffective, but trials are ongoing
- A safer alternative to immunizing with Aβ42 peptide might be immunization with DNA encoding for Aβ42 as the immune response differs: DNA immunizations shows in general a polarized T_H2 immune response that lacks the inflammatory profile seen in peptide immunizations

♦ Methods



♦ Results

BALB/c mice – 10 days after the last immunization

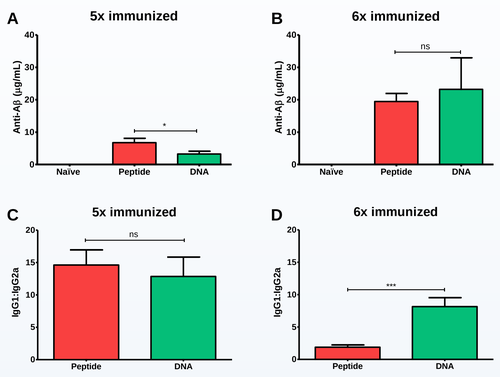


Figure 1: Anti-Aβ42 levels in plasma from peptide immunized and DNA immunized mice.
From BALB/c mice which had been immunized 5 times (A) or 6 times (B), anti-Aβ42 antibody levels were determined by ELISA. For 5x immunized mice, peptide immunized mice showed significantly higher antibody levels than DNA immunized mice (A). For 6x immunized mice, no significant differences were found between the two groups (B).
OD values were used to calculate the IgG1:IgG2a ratio for 5 times (C) and 6 times (D) immunized mice. In 5x immunized mice, DNA and peptide immunized mice showed a predominant IgG1 response indicative of a T helper 2 (T_H2) immune response (C). In the 6x immunized mice, the peptide mice had a low IgG1:IgG2a ratio indicating a mixed T helper 1 (T_H1) and T_H2 response (C). 6x DNA immunized mice showed significance for the expected T_H2 immune response (D). (ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, as indicated.)

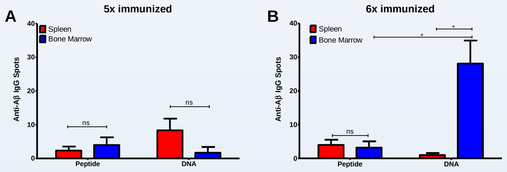


Figure 2: Anti-Aβ42 IgG producing cells in peptide immunized and DNA immunized mice.
From BALB/c mice which had been immunized 5 (A) or 6 (B) times with DNA Aβ42 trimer or human Aβ42 peptide, the numbers of anti-Aβ42 antibody producing cells were determined by ELISPOT. DNA immunized mice showed higher numbers of Aβ42 antibody producing cells in spleen after the 5th immunization; however, the difference was not significant due to the high standard deviation (A). After 6 immunizations, a significantly elevated number of anti-Aβ42 IgG producing cells were seen in the bone marrow of DNA immunized mice (B). Data were normalized using the number of CD19+ B cells in spleen versus bone marrow. (ns $p > 0.05$, * $p \leq 0.05$, as indicated.)

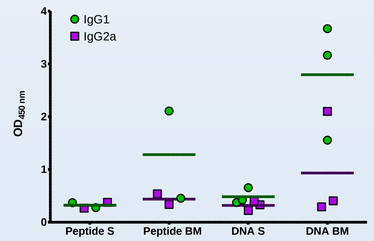


Figure 3: Anti-Aβ42 isotypes of *in-vitro* produced antibodies from cells of peptide and DNA immunized BALB/c mice upon re-stimulation.
From BALB/c mice which had been immunized with DNA Aβ42 trimer or human Aβ42 peptide, anti-Aβ42 antibody isotypes were determined by ELISA from supernatants of splenocytes and bone marrow cells cultured in PWM + anti-CD40 for 72 hours. Antibody levels measured were very low, but cells from peptide immunized mice secreted similar levels of IgG1 and IgG2a antibodies, indicating a mixed T_H1 and T_H2 immune response, while a clearly increased level of anti-Aβ42 IgG1 antibodies was found in supernatants of bone marrow cells of DNA immunized mice, indicating a T_H2 immune response. Data were normalized using the number of CD19+ B cells in spleen versus bone marrow.

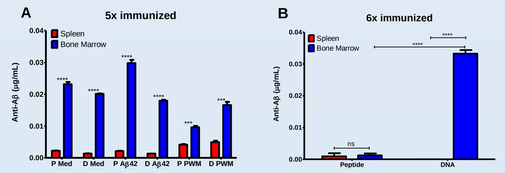


Figure 4: Analysis of the humoral response against Aβ42 *in-vitro* from cells of peptide (P) and DNA (D) immunized BALB/c mice upon re-stimulation.
For 5x immunized mice, anti-Aβ42 antibody titers in supernatants collected from spleen and bone marrow cells cultured in medium, Aβ42, and PWM for 72 hours were determined by anti-Aβ42 ELISA (A). Antibody production from bone marrow cells in both peptide and DNA immunized mice were significantly elevated in all culture conditions compared to the corresponding splenocyte culture supernatants. For 6x immunized mice, anti-Aβ42 antibody titers were determined from supernatants of spleen and bone marrow cells cultured in PWM for 72 hours (B). No significant differences were found in antibody levels of peptide immunized mice in either spleen or bone marrow. Bone marrow of DNA immunized mice contained significantly elevated levels of anti-Aβ42 antibodies. Data were normalized using the number of CD19+ B cells in spleen versus bone marrow. (ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, and **** $p \leq 0.0001$, as indicated.)

B6C3F1 mice – 4 months after the last immunization

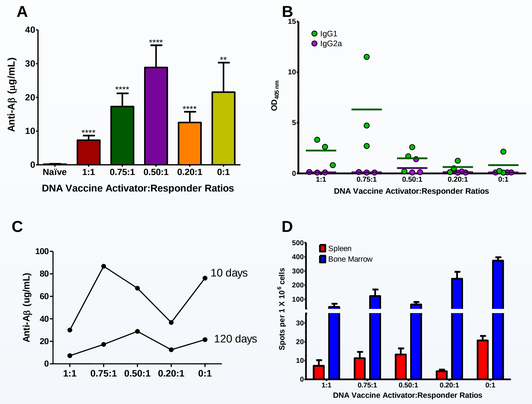


Figure 5: Analysis of the humoral response against Aβ42 in 8 times immunized B6C3F1 mice.
From B6C3F1 mice which had been immunized with various ratios of the double plasmid DNA construct (activator Gal4:responder Aβ42 trimer), anti-Aβ42 antibody titers were determined by ELISA. Antibody titers in plasma of DNA immunized mice were all significantly elevated compared to naïve mice (A). IgG isotypes were determined by ELISA (B) and optical density (OD) values were used to calculate the IgG1:IgG2a ratio. Comparing relative values of IgG1 and IgG2a in each group, mice immunized with 1:1, 0.75:1, 0.20:1, or 0:1 DNA activator: responder constructs showed significantly elevated IgG1 levels with ratios greater than 4.85 indicating a T_H2 response (data not shown). The antibody levels declined in all groups during the four month post-immunization period, but good antibody concentrations were still detectable in plasma (C). Numbers of anti-Aβ42 antibody producing cells in spleen and bone marrow were determined by ELISPOT (D). Data was normalized using the number of CD19+ B cells in spleen versus bone marrow. Bone marrow from DNA immunized mice showed higher levels of anti-Aβ42 IgG producing B cells compared to spleen, suggesting that the bone marrow may be the place where antibody producing plasma cells following DNA immunization reside. (ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, as indicated.)

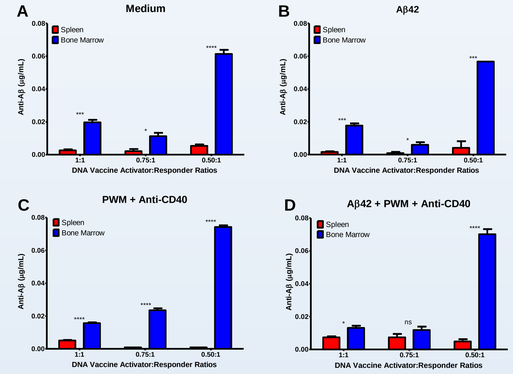


Figure 6: Anti-Aβ42 titers in supernatants of cells of bone marrow and spleen from DNA immunized B6C3F1 cultured for 72 hours with medium, Aβ42, PWM + anti-CD40, and PWM + anti-CD40 + Aβ42 mice.
Supernatants from bone marrow cells showed higher antibody production compared to samples from spleen cells. High antibody levels were found in medium culture conditions indicating that plasma cells do not need further activation for antibody secretion. Data were normalized using the number of CD19+ B cells in spleen versus bone marrow. (ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, and **** $p \leq 0.0001$, as indicated.)

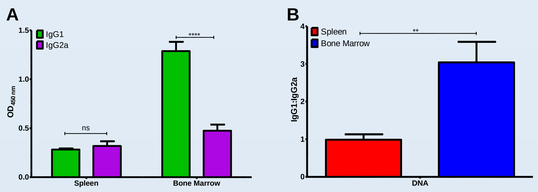


Figure 7: Anti-Aβ42 isotypes in supernatants of cells of bone marrow and spleen cultured with PWM ± anti-CD40 from DNA immunized B6C3F1 mice.
From B6C3F1 mice, anti-Aβ42 antibody isotypes were determined by ELISA from cell culture supernatants stimulated with PWM ± anti-CD40 for 72 hours (A). No significant differences were found between IgG1 and IgG2a isotypes in supernatants from spleens of DNA immunized mice, indicating a mixed T_H1 and T_H2 immune response. IgG1 levels were significantly elevated in supernatants from bone marrow of DNA immunized mice, indicating a T_H2 immune response. OD values were used to calculate the IgG1:IgG2a ratios which showed again that antibodies produced from bone marrow cells of DNA immunized mice have a high IgG1:IgG2a ratio whereas antibodies produced from spleen cells in the same mice have similar levels of IgG1 and IgG2a indicative of a mixed immune response (B). (ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, and **** $p \leq 0.0001$, as indicated.)

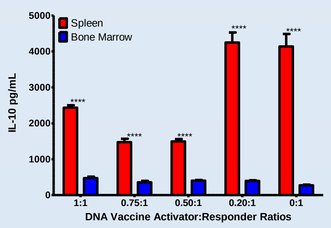


Figure 8: IL-10 levels in supernatants of cells of bone marrow and spleen from immunized B6C3F1 mice cultured with PWM ± anti-CD40 for 72 hours.
IL-10 levels from supernatants cultured with PWM ± anti-CD40 were determined by direct cytokine ELISA. Splenocytes in all culture conditions of DNA immunized mice showed significantly elevated levels of IL-10 compared to bone marrow cells. This is possibly showing that IL-10 in the DNA immunized mice is produced by T cells since the FACS profile showed very low levels of T cells in the bone marrow compared to spleen (data not shown) but this needs further testing.
(ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, and **** $p \leq 0.0001$, as indicated.)

♦ Results

- **Immune response in BALB/c mice shortly after last immunization (10 days)**
  - Plasma antibodies in peptide immunized mice were of a mixed T_H1 and T_H2 immune response, while plasma antibodies in DNA immunized mice were predominantly T_H2 indicative of a non-inflammatory humoral immune response in DNA immunized mice. Anti-Aβ42 antibody levels were similar in both types of immunizations. **Figure 1**
  - After *in-vitro* re-stimulation, in the 6x immunized mice, higher levels of anti-Aβ42 antibodies were produced from bone marrow cells of DNA immunized mice, which was not found for mice which had received 5 immunizations. From the antibody isotype analysis, we found a mixed immune response from cells of both spleen and bone marrow for the peptide immunized mice. Cells from spleens of DNA immunized mice showed a mixed immune response, whereas bone marrow cells from these mice showed a predominant T_H2 immune response. **Figures 2 and 3**
  - Elevated levels of anti-Aβ42 antibody production were found for cells from bone marrow of 6x DNA immunized mice, while cells from 6x peptide immunized mice had low levels of anti-Aβ42 antibody producing plasma cells in both spleen and bone marrow, suggesting that the DNA immunization may preferentially stimulate a strong antibody response with bone marrow plasma cells being the most responsive. **Figure 4**
- **Long term immune response in B6C3F1 mice (4 months)**
  - Following immunization, all ratios tested of the DNA Aβ42 trimer vaccine provided high titers of specific antibodies with a high IgG1:IgG2a ratio. Bone marrow was shown to be the predominant source of antibody producing plasma cells in this immunization protocol. **Figure 5**
  - Elevated levels of anti-Aβ42 antibody secretion was found in the bone marrow of DNA immunized mice after antigen and non-antigen specific stimulation, consistent with the fact that antigen specific stimulation is not necessary for the antibody production from long lived plasma cells. **Figure 6**
  - Isotyping for the antibodies produced from bone marrow cells showed the expected predominant T_H2 response, while antibody isotypes from the spleen showed a mixed immune response (**Figure 7)** similar to the results for the BALB/c mouse experiments (**Figure 3**).

♦ Open Questions

- Duration of antibody response from bone marrow
- Phenotypic characterizations for plasma cells from bone marrow compared to spleen after DNA immunization
- Which cells are the IL-10 producers following DNA Aβ42 trimer immunization? **Figure 8**

♦ References

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2. Qu B, Rosenberg RN, Li L, Boyer PJ, Johnston SA (2004). "Gene vaccination to bias the immune response to amyloid-beta peptide as therapy for Alzheimer disease." *Archives of Neurology* 61: 1859-1864.
3. Qu BX, Lambracht-Washington D, Fu M, Eagar TN, Stüve O, Rosenberg RN (2010). "Analysis of three plasmid systems for use in DNA A beta 42 immunization as therapy for Alzheimer's disease." *Vaccine* 28: 5280-5287.

♦ Acknowledgements

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