Amyloid Beta Nano Particle used to Sensitize Dendritic Cells as a Therapeutic Vaccine against Alzheimer's disease

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Abstract

The annual cost of Alzheimer's Disease (AD) is approximately $230 billion and it is estimated that the incidence of AD will increase three-fold if there is still no solution by the year 2050. Thus, there is a desperate need to develop and implement effective strategies for AD. Until now, FDA approved drugs could only temporally improve memory by increasing the neurotransmitters in the brain by inhibiting either the acetylcholine esterase activity or NMDA receptor. Therefore, results from data of all FDA approved drugs show that they could not stop or slow down the disease progress at all. Since Aβ accumulation and Tau phosphorylation are considered to be the major pathological factor, immunotherapy against pathological factors such as Aβ and Tau have emerged in the past 16 years. There has been evidence suggesting that an active vaccination strategy targeting the amyloid beta (Aβ) protein (i.e. Aβ 1-42) may have some efficacy, as stated by studies in a transgenic mouse model for AD. The history of immunotherapies against AD reveals that AD patients are old subjects with deteriorated immune systems, so a normal vaccine strategy will not work on this population unless strong adjuvants are used to stimulate and over prime the immune system. However, such adjuvants will lead to over activation of the immune system that induces an unwanted response. On the contrary, the passive immunotherapy relies on infused antibody, and antibody will not be able to have immune activate effects, so it will not be proper for long term treatment for AD patient. Thus, the best approach for effectively dealing with AD must be able to simultaneously target the pathological factor and address the impaired immune system for a longer period time. This can be achieved by using dendritic cells obtained from the patient and sensitizing the cells with a mutated form of Aβ. This allows for the subject's immune system to target the aggregated Aβ therefore reducing the symptoms of AD.

Materials and Methods

Major materials: All peptides used in this project were ordered from Biomer Tech Inc. (CA); Amyloid beta ELISA was assembled by our lab.

Methods:

DC preparation: Mouse bone marrow was removed from 7-11 week old female APP695/PS1 mouse. Detail methods is described by Cao et al. [7]

DC sensitization to peptide: On the second day of culturing, the medium was completely aspirated. Three ml of fresh DC culture medium was then added and kept in a CO2 incubator with 5% CO2. On the 4th day of culturing, the medium was partially changed and the cells treated as follows: 1ml/well old medium was aspirated and replaced by 1ml/well fresh DC culture medium containing 60μg/ml peptide (final concentration 20ug/ml). On the 8th day, DCs were harvested, washed twice, and counted to adjust to $5X10^6$ cells/ml.

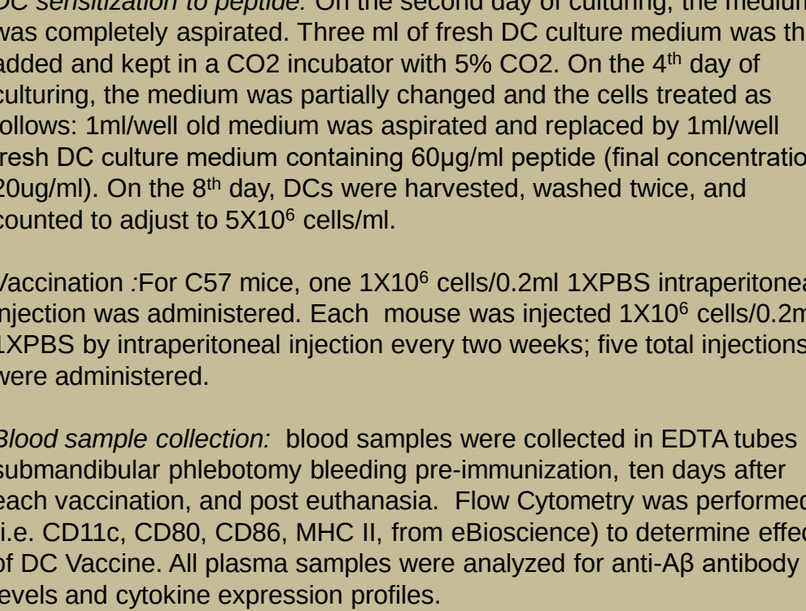
Vaccination :For C57 mice, one $1X10^6$ cells/0.2ml 1XPBS intraperitoneal injection was administered. Each mouse was injected $1X10^6$ cells/0.2ml 1XPBS by intraperitoneal injection every two weeks; five total injections were administered.

Blood sample collection: blood samples were collected in EDTA tubes by submandibular phlebotomy bleeding pre-immunization, ten days after each vaccination, and post euthanasia. Flow Cytometry was performed (i.e. CD11c, CD80, CD86, MHC II, from eBioscience) to determine effect of DC Vaccine. All plasma samples were analyzed for anti-Aβ antibody levels and cytokine expression profiles.

Antibody, cytokine detection is conducted by following the method published by Narba et al.

Results and Discussion

1. Aβ with muted T cell epitope altered the immunogenicity and broke tolerance



Aβ Peptide name	HLA-I affinity score
Wild type (PWT)	453.27
Flemish mutation (PFM=A21G)	453.27
Dutch mutation (PDM=E22Q)	925.042
Flemish plus Dutch (PFDM=A21G+E22Q)	925.042
New mutation1 (E22W)	6937.812
New mutation2 (V24G)	0.486

FIGURE 1. Western Blot results of the aggregation assay of Aβ peptide derivatives and their fragments. The results show that Mutation in T cell epitope can affect the aggregation of Aβ and fragmented Aβ has the similar performance as the full length of Aβ.

Results and Discussion

2. Aβ with a mutated T cell epitope is required to sensitize DCs and not for Sub-Q vaccine

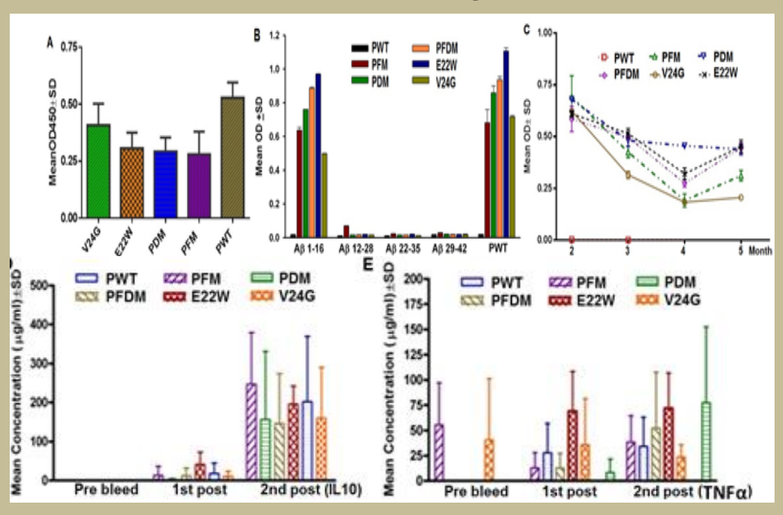


Figure2. Comparison of Peptide sensitized DCs vaccine to Sub-Q injection vaccine. The mutated T cell epitope is required to sensitize DCs for immune response, and wild type Aβ doesn't have the ability to induce antibody response when applied as DCs vaccine. Importantly, wild type and mutated peptide have the same antibody response while delivered by Sub-Q injection. The DCs and Langerhans cells have different function in antigen presentation.

3. Our DCs vaccines target on oligomer with an anti-inflammation response, but the whole T cell epitope is required for antibody induction

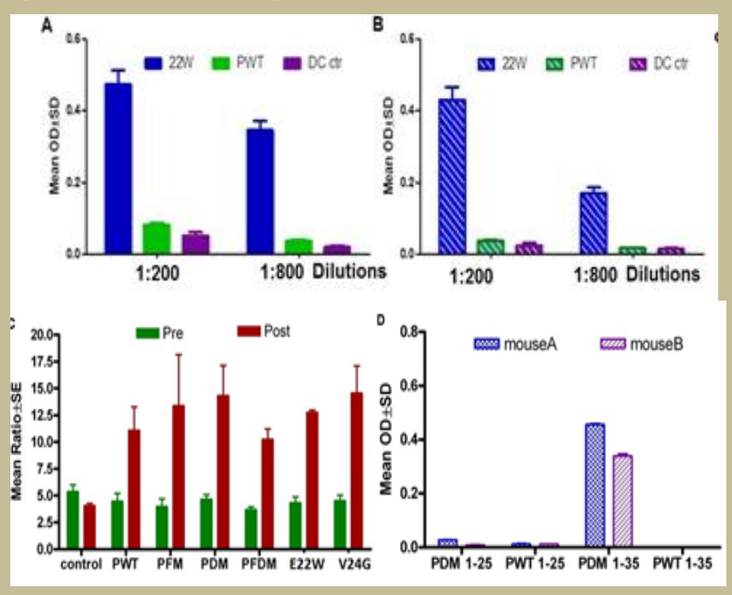


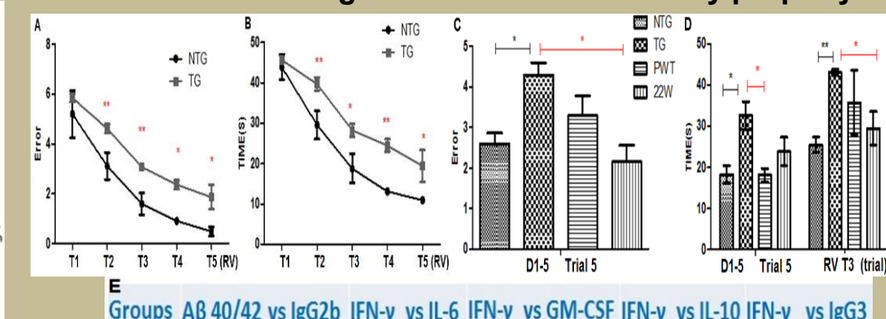
Figure 3 Antibody response of peptide sensitized DCs vaccine to Aβ and aggregated Aβ: The entire T cell epitope of Aβ is required for the mutated peptide to induce antibody response, and the DCs vaccine drives to anti-inflammation response and Preaggregation of mutated Aβ can be used as antigen to sensitize DCs and the preparation can induce oligomeric specific antibody without inducing inflammation, so develop a oligomeric specific DCs vaccine is possible.

4. The DCs vaccine induced antibody response in immunosenescence mice.

Figure 4 Immune senescence evidence and old DCs can still be sensitized by our mutated peptide as vaccine. These results imply that E22W sensitized DC can stimulate CD8+ and increase the function of the cells. Old mice have weaker immune systems therefore, the mutated peptide DC vaccine is preferred. The vaccine also may contain anti-infection activity.

Results and Discussion

5. The DCs vaccine improved memory in old APP/PS1 mice without losing DC's immunomodulatory property



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Groups	Aβ 40/42 vs IgG2b	IFN-γ vs IL-6	IFN-γ vs GM-CSF	IFN-γ vs IL-10	IFN-γ vs IgG3
Control		***	***	***	
PWT					**
E22W	***	***	***	***	

Figure 5. Analysis of behavioral tests. Mutant peptide sensitized DCs vaccine can improve memory in old APP/PS1 mice without losing DC's immune function. E22W sensitized DCs used as vaccine doesn't change the immunomodulatory property of DCs cells, so this type of DC vaccine will be a better approach for AD treatment.

6. Co-polymer encapsulated Aβ fragment can induce oligomeric specific antibody response

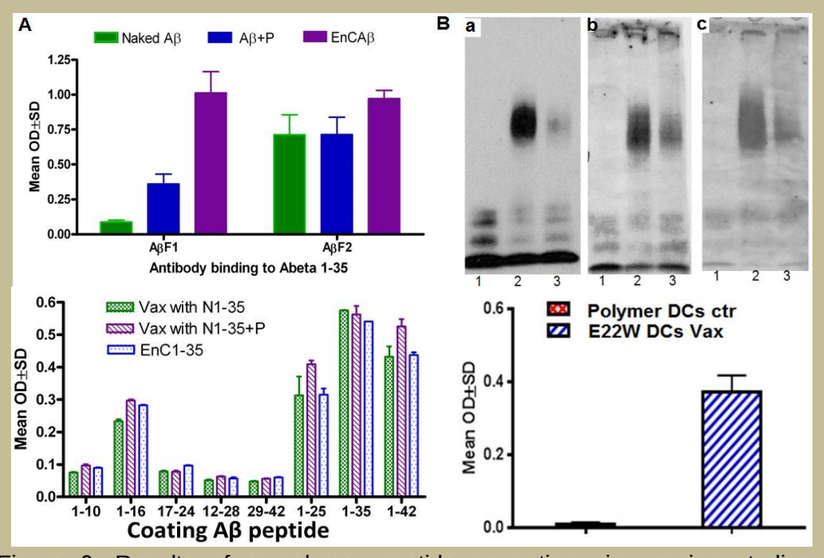

Figure 6. Results of co-polymer peptide as antigen in vaccine studies. Indicate that Co-polymer encapsulation can enhance the antigenicity and change the solubility in aqua solution without modifying the B cell epitope. Also, the encapsulation can provide microenvironment to favor the formation of oligomer Aβ to assist the generation of anti-oligomer Aβ antibody when used as vaccine. Importantly, the encapsulated mutant peptide formulation can effectively sensitize the dendritic cell and induce antibody response.

Conclusion

The provocative experiments summarized in the results and discussion section indicate several important conclusions: (a) only Aβ peptides with mutations in the T cell epitope can sensitize DCs as vaccine and the preparation can generate a potentially important antibody response; (b) Preaggreggrated Aβ peptide with mutation sensitized DCs target the biologically relevant oligomeric form of Aβ and DCs vaccine mediate amelioration of memory deficits that occur a murine AD Tg model; (c) the Aβ fragment with whole T cell epitope is easier to synthesize with fewer batch to batch variations and functions in these studies proved the similarly to Aβ 1-42; (d) co-polymer encapsulated peptide can generate oligomer specific anti-Aβ antibody and the preparation can sensitized DCs and use as vaccination; (e) Mutant peptide sensitized. These results provide the foundation for future studies using the mutated peptide to sensitize DCs for the generation of AD therapy.

References

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