



2011 Research Grant Program Winning Abstract

Phenotypic and Functional Characterization of T Cell Subsets Generated in DNA A β 42 Trimer Immunized Mice to Determine Safety in Immunotherapy for Alzheimer's Disease

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Alzheimer's disease (AD) is the most common form of dementia found in the elderly. No cure or effective treatment for this devastating disease has been found to date. AD leads to a complete loss of memory and to loss of the ability to function independently. Estimates propose that up to 4 million Americans currently have AD, with costs to society of up to \$100 billion per year. The possibility of an immune-mediated approach as a preventive or therapeutic intervention that reduces amyloid plaques without causing brain inflammation has great impact for future clinical use.

AD has been strongly associated with buildup of the self peptide Amyloid beta 1-42 (A β 42) in the brain. Immunizations with A β 42 peptide have been shown to reduce amyloid burden in the brain and improve cognitive function in transgenic mouse models for AD. In humans, however, A β 42 peptide vaccination was discontinued because of the occurrence of meningoencephalitis in 6% of patients due to a Th1 autoimmune response. A follow-up of a few study participants showed that A β 42 peptide vaccination did lead to a reduction in plaque load in patients who had been treated with A β 42 peptide compared to patients who had been given a placebo control, providing proof that it is possible to remove brain amyloid by immunotherapy.

Genetic immunization, in which the immunizing agent is DNA encoding A β 42, has great potential to treat or prevent AD because the immune response differs in several important points from the immune response elicited by peptide immunization. Our previous findings showed a predominantly Th2-type antibody response and the disappearance of an A β 42 specific T-cell response at later immunization time points. A complete characterization of cellular components of the immune response against A β 42 is needed for an understanding of how an inflammatory cellular response against the self antigen A β 42 can be avoided by use of this DNA A β 42 immunization approach. Our hypothesis is that the polarized T helper 2 (Th2) cellular immune response following genetic immunization leads only to the production of a beneficial and therapeutic antibody response, and we hypothesize that the low level of T-cell proliferation in DNA A β 42 trimer immunized mice is due to increased numbers of regulatory T cells (Tregs) that dampen the cellular response found at later immunization time points.

To test our hypothesis we are performing phenotypic and functional analyses of the immune response to DNA A β 42 trimer and the comparison to the immune response elicited by A β 42 peptide. In this project we will focus on the analysis of a regulatory immune response, and we have developed the following specific aims:

Specific Aim 1: Phenotypic analysis of the respective T-cell populations in our immunized mouse models using multicolor flow cytometry. We will characterize the cells



via cell surface markers and intracellular staining for expression of cytokines and signature transcription factors, eg, expression of the transcription factor FoxP3 is the hallmark of regulatory T cells ($CD4^+CD25^+FoxP3^+$). We hypothesize that regulatory T cells play a significant role in the dampening of the immune response following DNA A β 42 trimer immunizations and we expect to find increased numbers of these cells in DNA immunized mice in comparison to peptide-immunized mice. Since one subpopulation of effector/memory FoxP3 $^+$ T cells develops in the thymic medulla and a second subpopulation is thymus independent, we will further separate the Tregs into natural Tregs (thymic development) and induced Tregs (thymus independent). Both lineages display a distinct activated phenotype (Helios negative and Helios positive) and are unique in their capacity to mediate suppression of T-cell activation. $CD4^+CD25^+FoxP3^+$ T cells may be important for tolerance by rapidly suppressing immunity to self-Ags. These studies would further the benefits of our immunization approach, since they would prove that the danger of autoimmune reactions is downregulated by this particular cell population.

Specific Aim 2: Functional analysis of the Treg subset from DNA A β 42 trimer immunized mice. Here we will evaluate the potential of Tregs resulting from DNA immunization to downregulate the cellular immune response to A β 42 in co-culture experiments with antigen specific responder cells from peptide-immunized mice. The Tregs ($CD4^+CD25^+$) will be obtained using the BD FACSAria™ cell sorter (BD Biosciences) and titrated into the cultures of CFSE labeled responder cells (ratios 1:1, 0.25:1, 0.1:1). Inhibition of the CFSE proliferation will be analyzed by flow cytometry.

Specific Aim 3: We hypothesize that the absence of a CD8 T-cell response in the DNA A β 42 trimer immunized mice is due to increased Tregs in our model. To test whether increased numbers of Tregs in our model are indeed responsible for the lack of A β 42 specific CD8 T cells in DNA-immunized mice, we would treat the mice prior to the immunizations with depleting CD25 antibodies (antibody clone PC61, BD Biosciences), since this had been described to be effective in both removing Tregs and improving CD8 T-cell responses. The read-out of these experiments will be the analysis of A β 42 specific CD8 T-cell proliferation by CFSE dilutions and analysis of the frequencies of potential cytotoxic T cells with the CD107 mobilization assay. Both are established flow cytometry based methods in our lab.

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