

# Improved CD4 T cell Responses against Hepatitis C virus Non Structural Protein NS3 in DR0401 Transgenic Mice

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Hepatitis C virus (HCV) infection is correlated with the risk of developing liver cirrhosis and hepatocellular carcinoma. Worldwide it is estimated that more than 170 million are chronic carriers and no prophylactic vaccine exist nor an effective therapeutic treatment.

Strong CD4 and CD8 T cell responses against the non structural protein NS3 have been shown to be important for T cell mediated immunity in humans who have obtained viral clearance after an acute HCV infection. In order to design an effective vaccine against hepatitis C virus (HCV), we coupled NS3 (genotype 1b) to invariant chain (Ii), the chaperone protein for MHC-II molecules, and expressed the construct in an adenoviral vector. This was done in order to improve the CD4 T cell responses as we anticipate that this specific guiding to the endosomes will induce a strong NS3 specific Th1 polarised CD4 T cell response (IFN $\gamma$ ) that will fight the virus directly as well as support development of long term memory of NS3 specific CD8<sup>+</sup> T cells. The vaccine was tested in a humanized mouse model ( $\alpha\beta^{-/-}$ , DR0401<sup>+</sup>, human CD4<sup>+</sup> transgenic mice) in an attempt to mimic the response seen in humans.

Mice were vaccinated with recombinant NS3 in Complete Freud's Adjuvant as prime and either rNS3, AdNS3 or AdIiNS3 as boost three weeks later. Mice were sacrificed 10-12 days after the boost and spleenocytes were analyzed for IFN $\gamma$ , TNF or IL2 cytokine production by intracellular flow cytometry. The prime boost vaccination with recombinant NS3 followed by AdIiNS3 increased the cytokine response both in the CD4 T cells compared to the other two vaccination groups. The same CD4 T cell epitopes found in this study are the same epitopes as seen in a DR0401 positive patient that recovered completely from an HCV infection.

Previously NS3 specific CD4 T cell hybridomas were generated from DR0401 transgenic mice by vaccination with recombinant NS3 protein and six CD4 epitopes were identified. Three of these T cell hybridomas recognizing three different CD4 epitopes were used in this study to evaluate the capacity of dendritic cells (DCs) transduced with either AdNS3 or AdIiNS3 to stimulate the CD4 T cell hybridomas. Only the DCs that were transduced with AdIiNS3 could activate the CD4 T cell hybridomas.

In conclusion, the linkage of Ii to NS3 significantly improved the CD4 T cell response and gave a stronger and broader CD4 T cell response compared to the other two vaccinations.