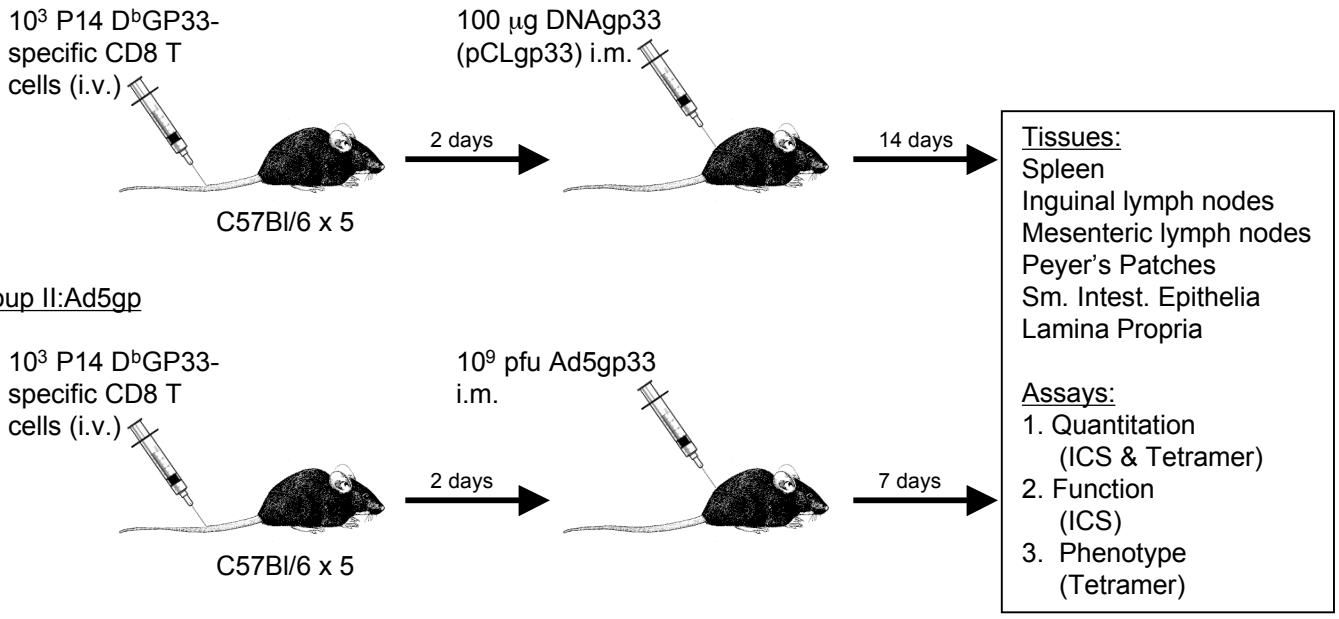
MCEL2008001: Evaluation of the peak GP33 CD8 T cell response to Ad5gp and DNAgp vaccination in C57Bl/6 mice

Mouse Immunology Laboratory
Collaboration for AIDS Vaccine Discovery

Experimental Design:

Group I:DNAgp



Group II:Ad5gp

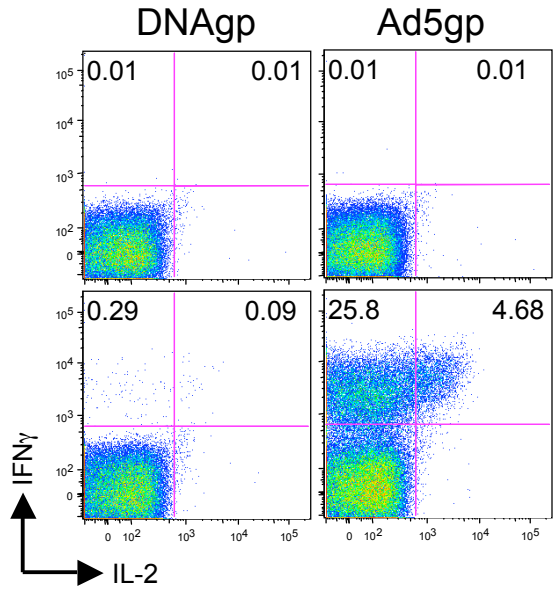
Results/Conclusions:

Ad5gp immunization resulted in greater (10-100 fold increased) overall CD8 T cell responses than did DNAgp immunization, especially in the spleen and draining lymph node. However, CD8 T cell responses to the gp33 epitope in the Peyer's patches were similar between both Ad5 and DNA immunized mice. These results indicate that DNA immunization may result in the preferential induction of effector responses at this mucosal site, and it is tempting to speculate that human studies, which show that DNA immunization results in undetectable responses when systemic T cell compartments such as the peripheral blood, may be underestimating responses to the vaccine at other sites. However, since there are known differences between humans and mice in the cell type expression pattern of TLR9, subsequent experiments will be done to evaluate the elicitation of gp33 responses to these vaccines in wt and $TLR9^{-/-}$ mice, or in mice that we have developed for "humanized" expression of TLR9; these mice express human TLR9 only plasmacytoid dendritic cells, similar to the expression pattern seen in humans, and have been developed on a mouse TLR9-/- background.

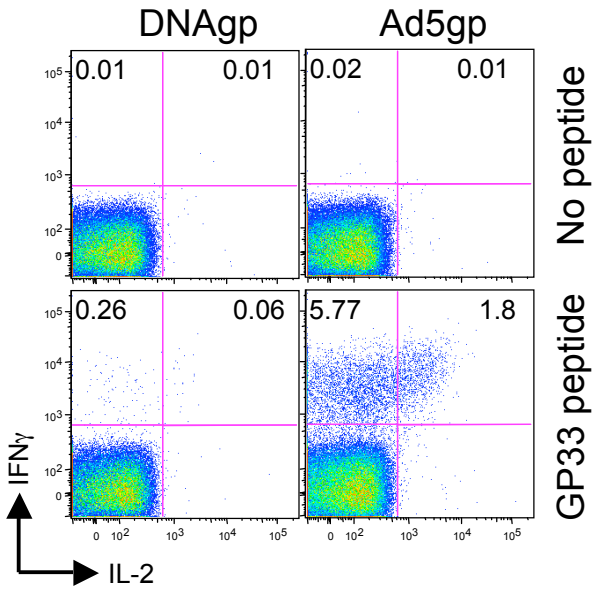
Phenotypic and functional analysis of T cell responses in this experiment showed some differences in cell surface expression and intracellular cytokine production between gp33 responses in Ad5 and DNA immunized mice. In particular, although the overall responses was lower, there was an increased proportion of IL-2 producing cells in spleens from DNA immunized mice. In addition, there was an percentage of D^bGP33 $tetramer^+$ cells that were $CD62L^{lo}$ in spleens and draining lymph nodes from Ad5 immunized mice, but an increased fraction of $CCR9^{hi}$, $\alpha4\beta7$ $integrin^{hi}$ cells in mucosal tissues from DNA immunized mice. However, conclusions from such experiments must be tempered given the different time points for the peak of the DNA and Ad5 responses. Subsequent experiments should evaluate the phenotype of responding CD8 T cells in DNA and Ad5 immunized mice at the same time points.

I. Intracellular cytokine staining

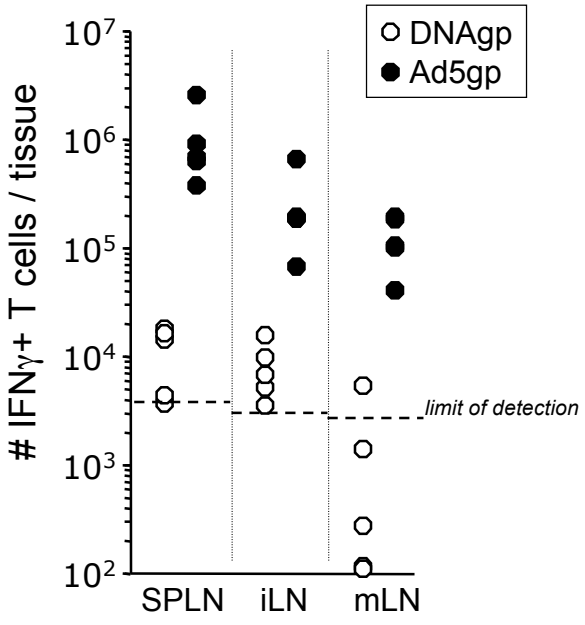
A. Representative staining (splenocytes gated on CD8+)



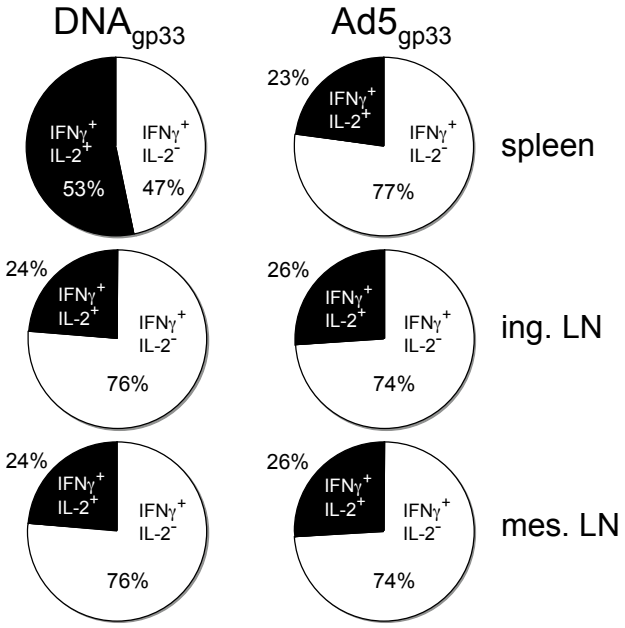
B. Representative staining (inguinal LN gated on CD8+)



C. Quantitation of GP33-specific cells by ICS

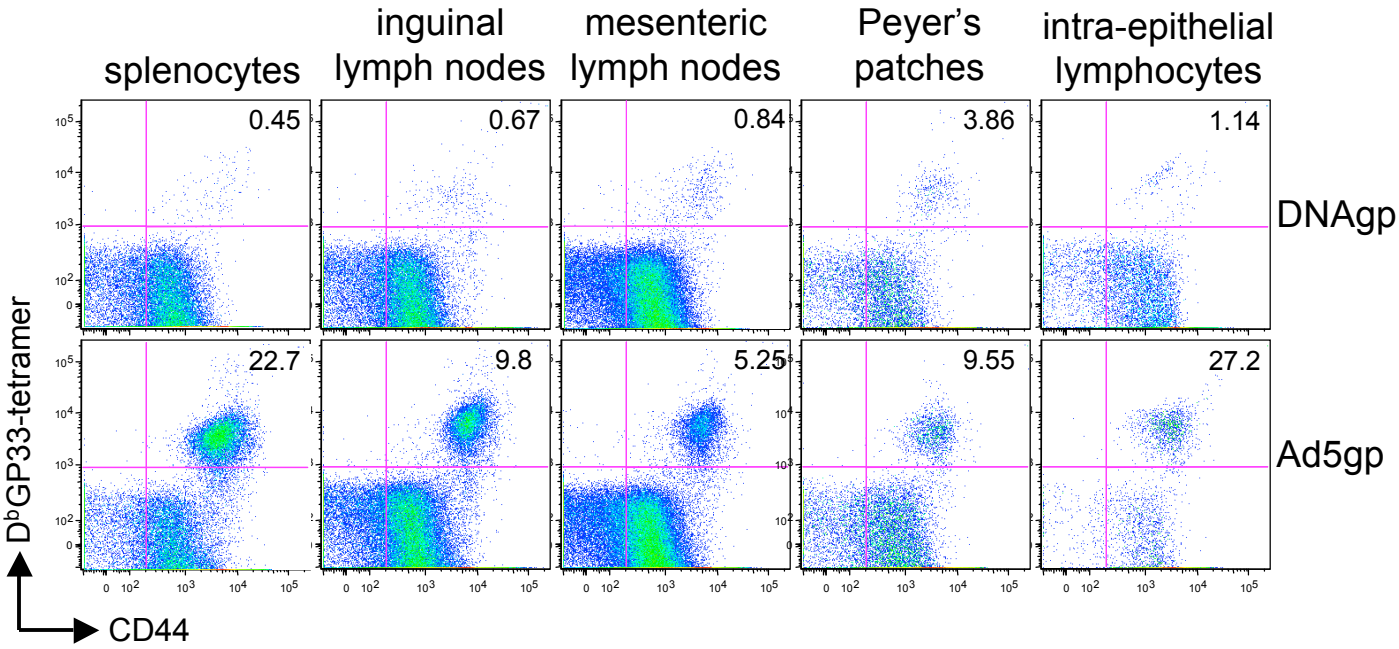


D. Functionality of GP33-specific cells by ICS

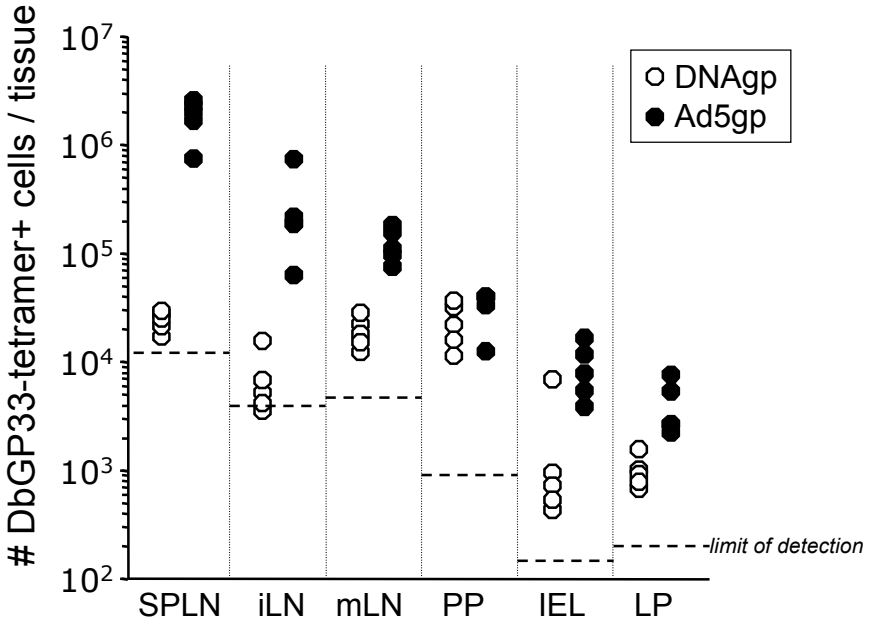


II. Tetramer staining

A. Representative staining (gated on CD8⁺ cells)



B. Quantitation of D^{b}GP33-specific cells by tetramer staining



C. Phenotypic analysis (gated on D^bGP33 tetramer$^+CD8^+$ cells)