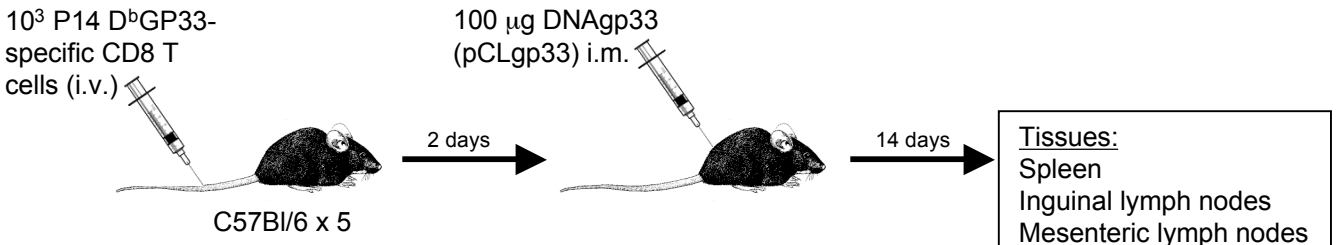
MCEL2008001: Evaluation of the peak GP33 CD8 T cell response to Ad5gp and DNAgp vaccination in C57Bl/6 mice

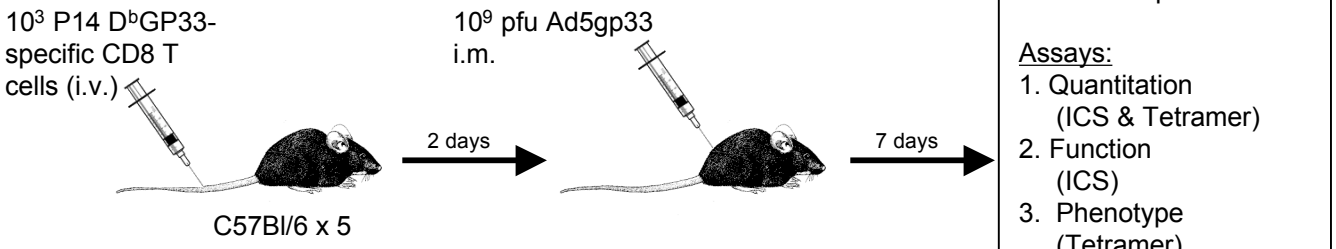
Mouse Immunology Laboratory Collaboration for AIDS Vaccine Discovery

Experimental Design:

Group I:DNAgp



Group II:Ad5gp



Tissues:
Spleen
Inguinal lymph nodes
Mesenteric lymph nodes
Peyer's Patches
Sm. Intest. Epithelia
Lamina Propria

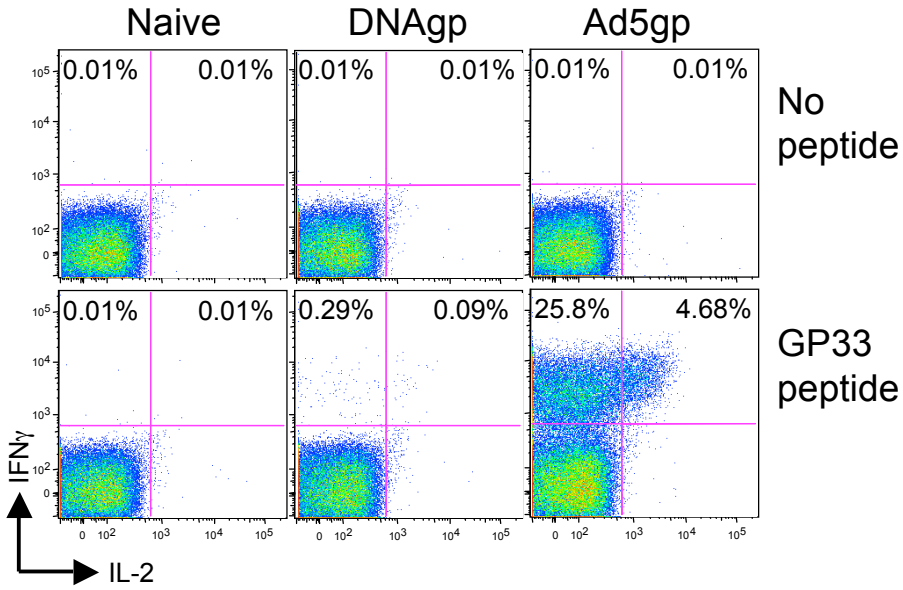
Assays:
1. Quantitation (ICS & Tetramer)
2. Function (ICS)
3. Phenotype (Tetramer)

This initial experiment is designed to evaluate the magnitude of the peak CD8 T cell response to a model antigen (LCMV-GP) expressed by either a DNA vaccine (DNAgp) or an Ad5 vaccine (Ad5gp). In order to visualize the responding population, naive P14 cells, specific for the gp33-41 epitope, were transferred 2 days prior to immunization. The dose of each vaccine used for immunizations was chosen based on previous experience with these vaccines indicating this to be the optimal dose. The time points for analysis of the peak of the T cell response in both vaccination strategies were chosen based on data from our lab as well as previously published data from others indicating that the peak of the DNA response occurs at 14 days and the peak of the Ad5 response occurs at 7 days post immunization. Systemic immune compartments and mucosal immune sites were sampled to determine the relative ability of these different vaccines to induce responses at these sites.

I. Intracellular cytokine staining (ICS)

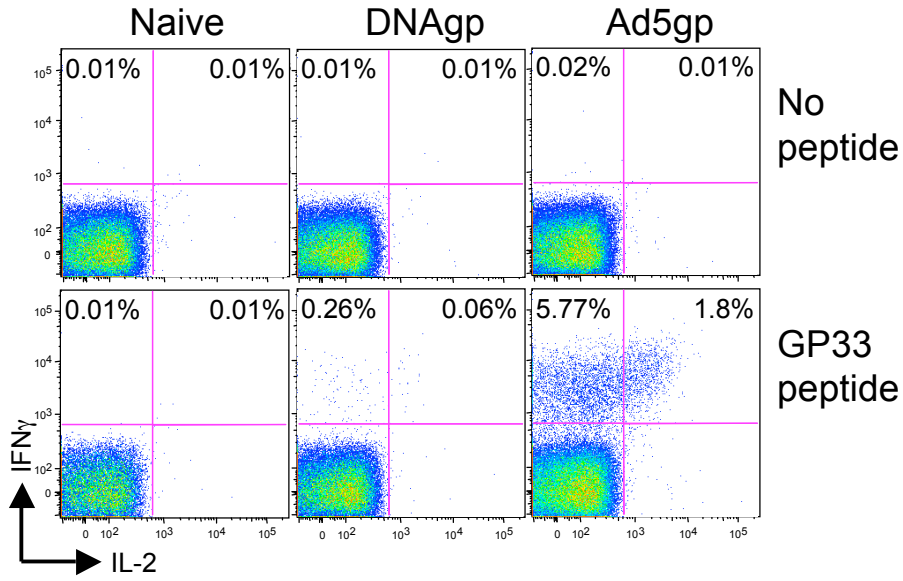
A. Representative intracellular cytokine staining of splenocytes (gated on CD8⁺)

Single cell suspensions of splenocytes were left unstimulated or restimulated with the LCMV gp33-41 peptide for 5 hrs in the presence of Brefeldin A then stained in for CD8, followed by staining for intracellular IFNγ and IL-2. FACS plots are gated on CD8⁺ T cells. Naïve=non-immunized B6 mouse.



B. Representative intracellular cytokine staining of inguinal lymph node cells (gated on CD8⁺)

Single cell suspensions of inguinal lymph node cells were left unstimulated or restimulated with the LCMV gp33-41 peptide for 5 hrs in the presence of Brefeldin A then stained for CD8 and intracellular IFNγ and IL-2 as above. FACS plots are gated on CD8⁺ T cells. Naïve=non-immunized B6 mouse.



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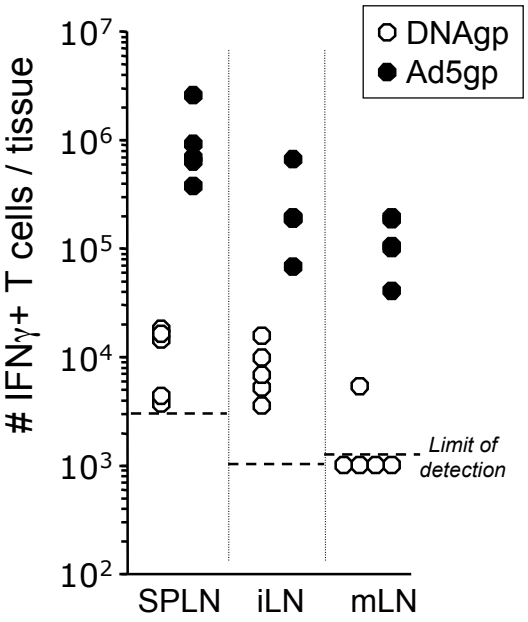
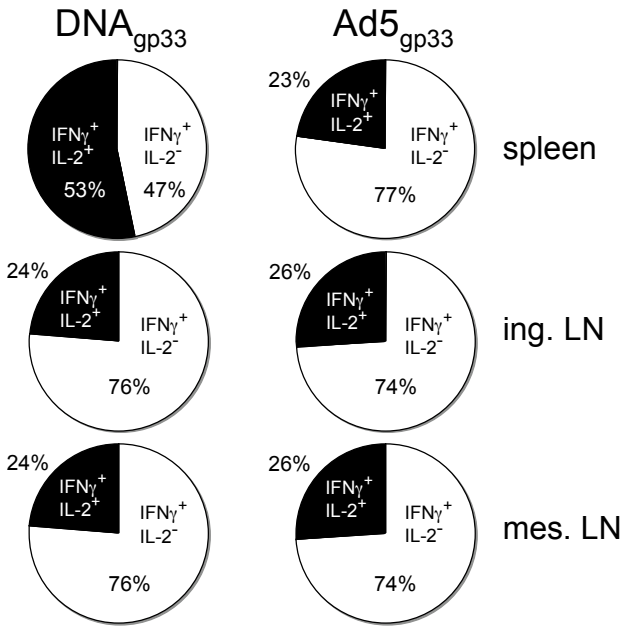
C. Quantitation of GP33-specific cells by ICS

Number of IFNγ+ cells is derived from cell recovery and % CD8+IFNγ+ data from ICS. SPLN=spleen, iLN=inguinal LN, mLN=mesenteric LN. Limit of detection is based on staining of cells from non-immunized mice



D. Functionality of GP33-specific cells by ICS

Percentages are of total IFNγ+ cells. ing.=inguinal, mes.=mesenteric. Functionality of responses in the mesenteric lymph nodes after DNA immunization are based on the single mouse in which a response was detected.



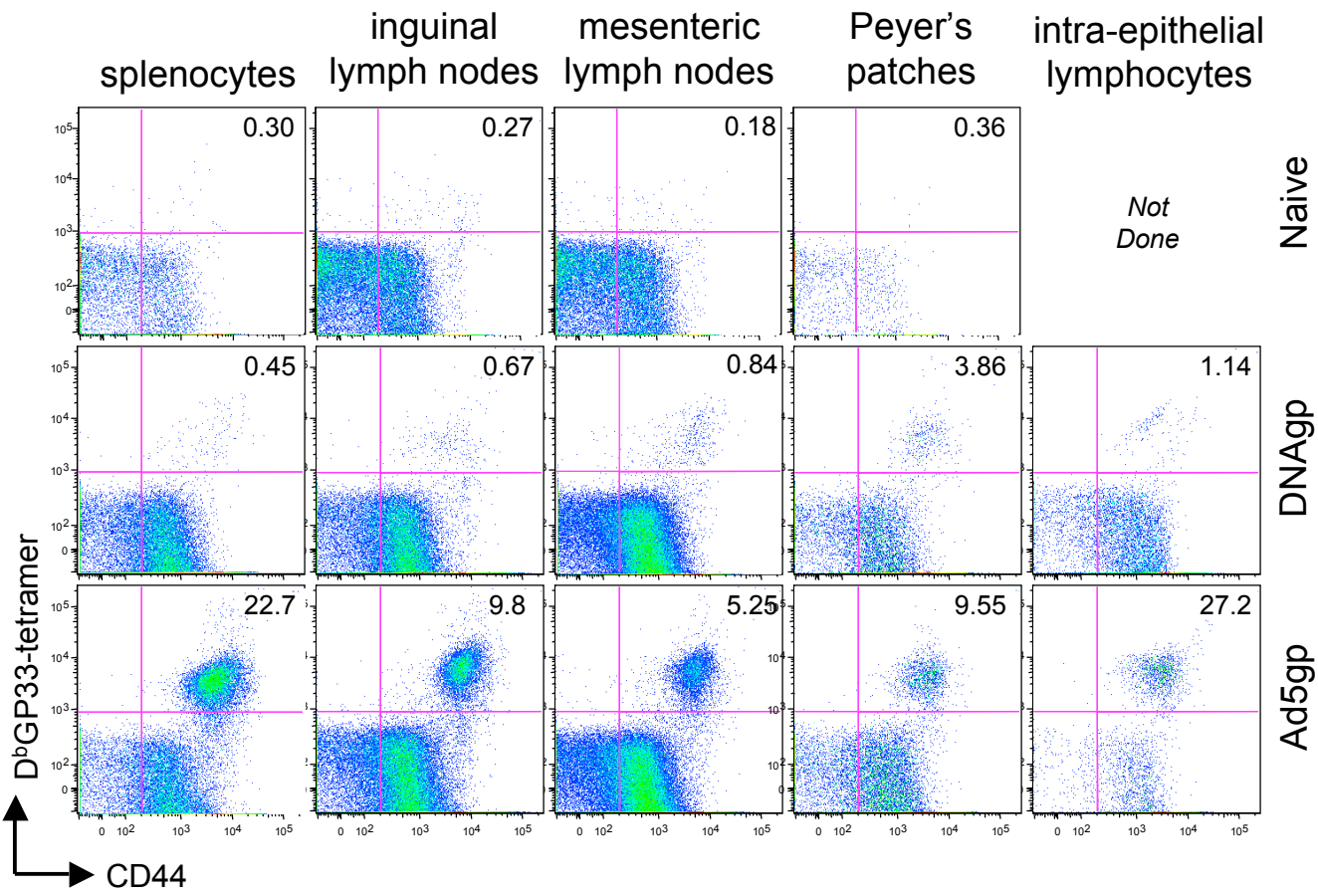
Summary Intracellular cytokine staining:

Ad5gp immunization resulted in ~100-fold greater overall CD8 T cell responses to the GP33-epitope than did DNAgp immunization in the spleen and draining lymph nodes, as detected by intracellular cytokine staining. However, responses were detectable above background levels in DNA immunized mice after a single immunization. In addition, responding CD8 T cells from the spleens of DNA immunized mice had a higher proportion of cells that were able to produce both IFNγ and IL-2 compared to cells from Ad5 immunized mice. This difference was not observed in other sites.

II. MHC tetramer staining:

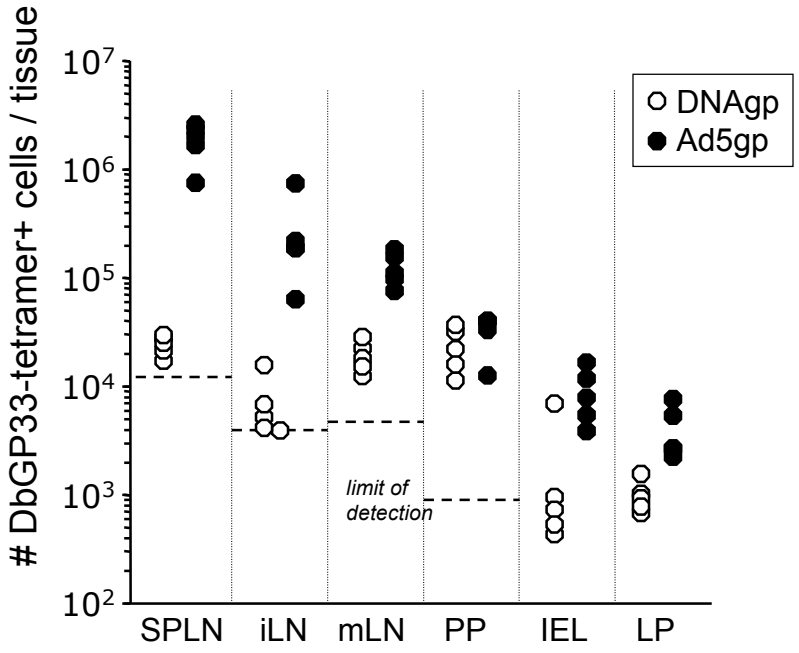
A. Representative tetramer staining of splenocytes (gated on $CD8^+$ cells)

Single cell suspensions of splenocytes from non-immunized (naïve), or from immunized mice at 14 days post DNAgp immunization or 7 days post Ad5gp immunization, were stained with the D^bGP33 tetramer and antibodies to CD8α and the activation marker CD44. FACS plots are gated on $CD8^+$ T cells. Not enough cells were recovered from the gut epithelia or lamina propria of non-immunized (naïve) mice for analysis.



B. Quantitation of D^bGP33-specific cells by tetramer staining

Absolute numbers of D^bGP33-specific CD8 T cells were determined based on cell recoveries and %$CD8^+$ and $Tetramer^+$ cells. SPLN=spleen, iLN=inguinal lymph nodes, mLN=mesenteric lymph nodes, PP=peyer's patches, IEL=intraepithelial lymphocytes, LP=lamina propria. Limit of detection is based on staining of cells from non-immunized (naïve) mice.



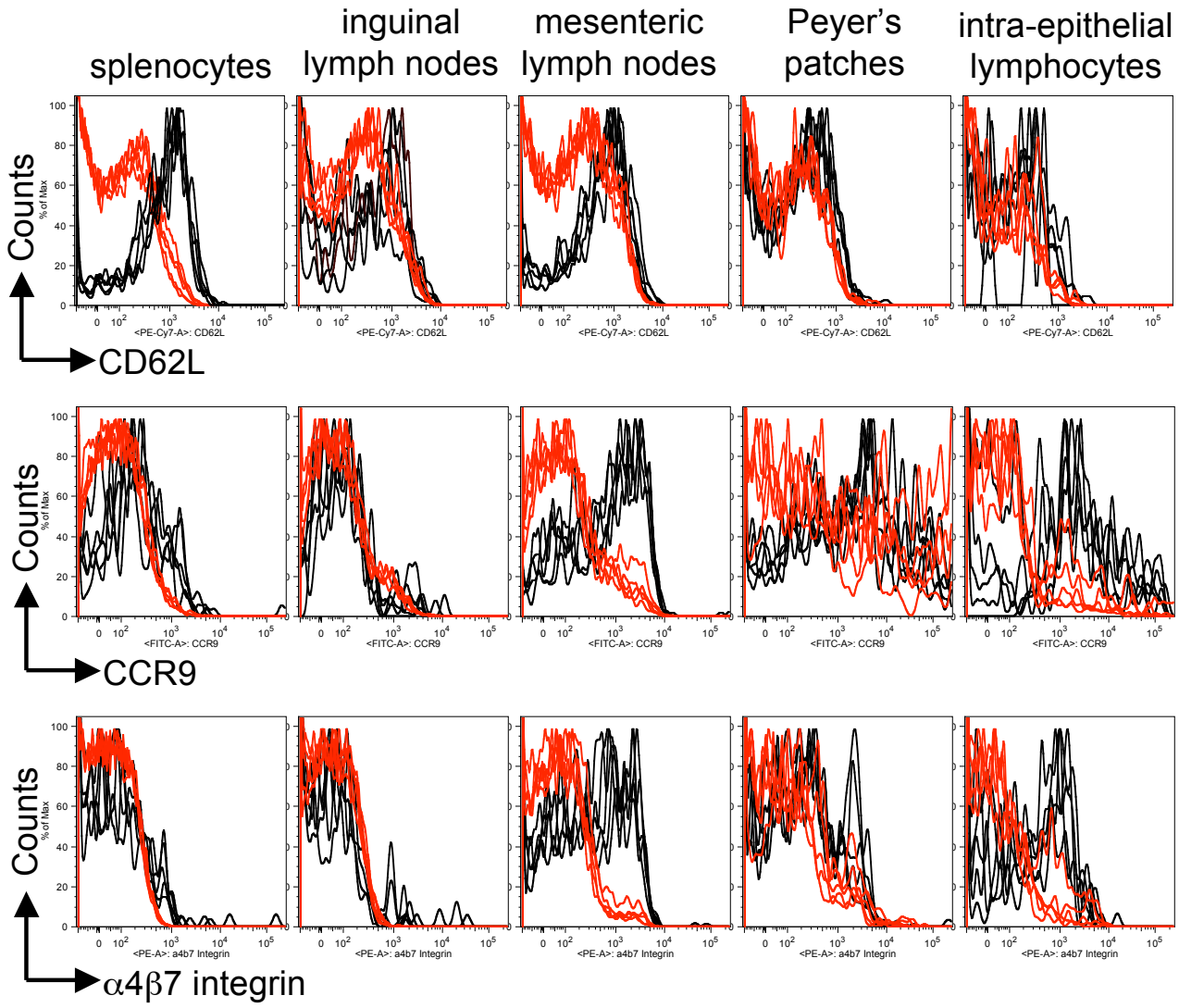
Summary Tetramer staining:

Similar to results from ICS, tetramer analysis revealed ~100 fold higher CD8 T cell responses in Ad5 immunized mice compared to DNA immunized mice in the spleen and draining lymph nodes. However, although overall responses were higher in Ad5 immunized mice, 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 that localize to mucosal sites. This may explain why DNA immunization in human studies results in priming of memory cells that can be detected during boosting but which are otherwise undetectable when systemic compartments such as the peripheral blood are analyzed; responses to the vaccine may preferentially localize to mucosal sites such as Peyer's patches.

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

Single cell suspensions of cells from the indicated sites were stained with the D^bGP33 tetramer and antibodies to CD8α and the indicated protein. Samples are gated on $CD8^+D^bGP33$ tetramer$^+$ cells.

Day 14 DNA_{gp}
Day 7 $Ad5_{gp}$



Summary phenotype analysis:

Although overall responses were larger in Ad5 immunized mice, there were a higher proportion of $CD62L^{hi}$ tetramer$^+$ cells in DNAgp immunized mice than in Ad5gp immunized mice. Additionally, DNA immunization resulted in a higher proportion of $CCR9^{hi}$ $\alpha4\alpha7$ integrin^{hi} tetramer$^+$ cells in mucosal sites such as the mesenteric lymph nodes. These differences may be due to differences in the timing of the peak of the responses to these two immunization strategies or be a result of different levels of antigen stimulation during DNA and Ad5 immunization resulting in different levels of effector T cell differentiation. Alternatively, these data suggest that DNA immunization may elicit CD8 T cell responses that preferentially retain CD62L expression and/or the preferential recruitment or retention of $CCR9^{hi}$ $\alpha4\alpha7$ integrin^{hi} cells at mucosal sites.