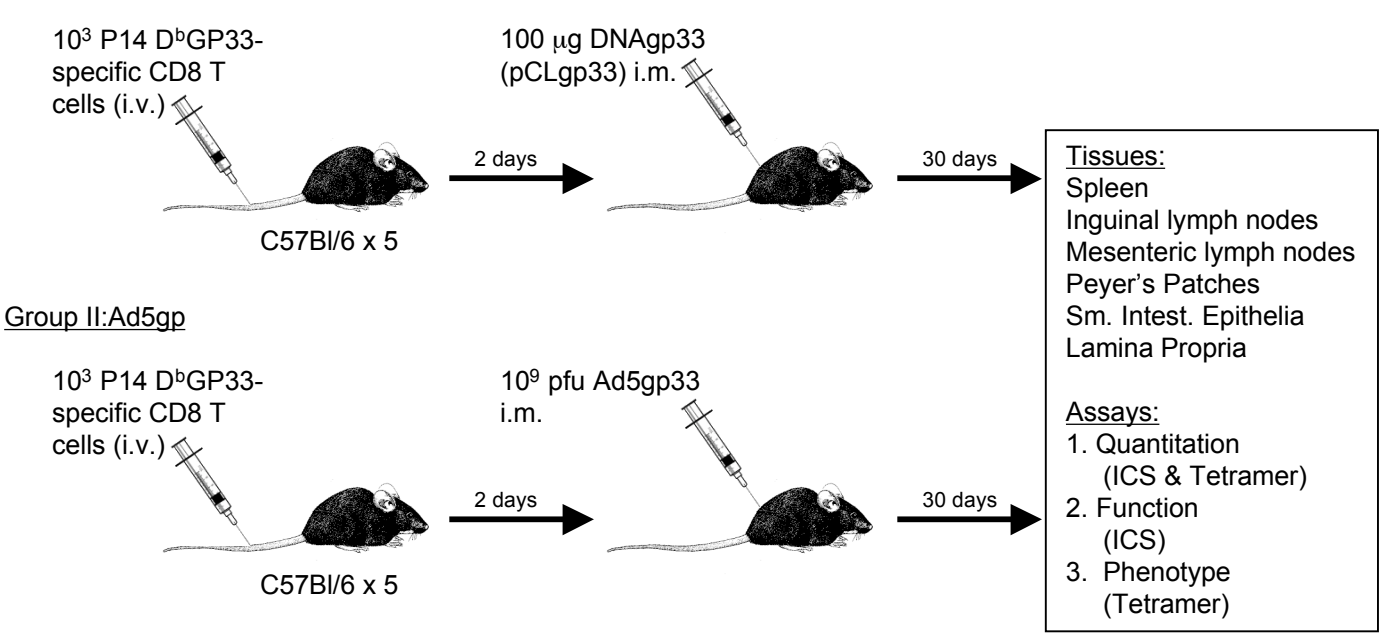
MCEL2008002: Evaluation of the memory GP33 CD8 T cell response to Ad5gp and DNAgp vaccination in C57Bl/6 mice

Mouse Immunology Laboratory Collaboration for AIDS Vaccine Discovery

Experimental Design:

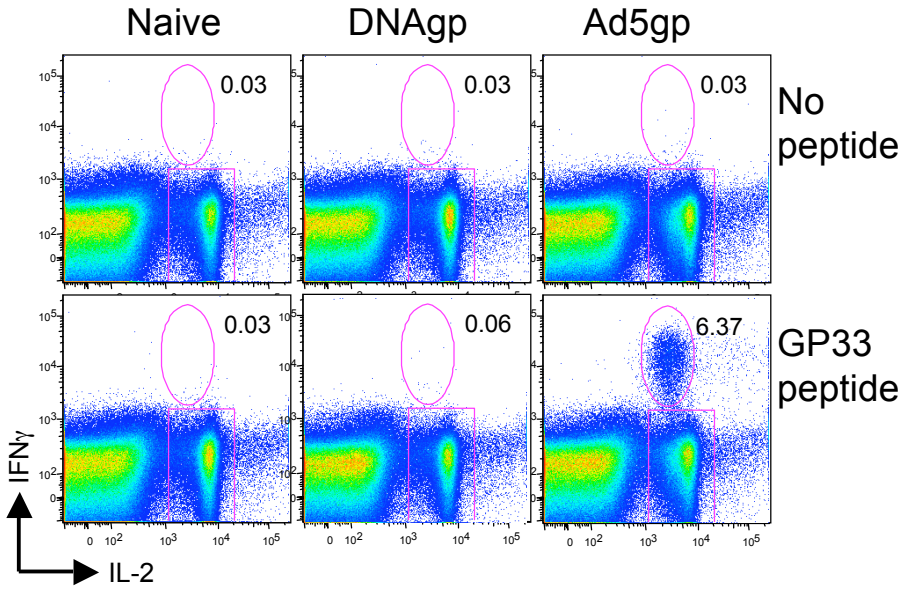


This experiment is designed to evaluate the magnitude of the memory CD8 T cell response to a model antigen (LCMV-GP) in mice immunized with either a DNA vaccine (DNAgp) or an Ad5 vaccine (Ad5gp) expressing this immunogen. In order to aid in tracking the responding population, congenically marked naive 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 memory 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 memory levels are established at >28 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)

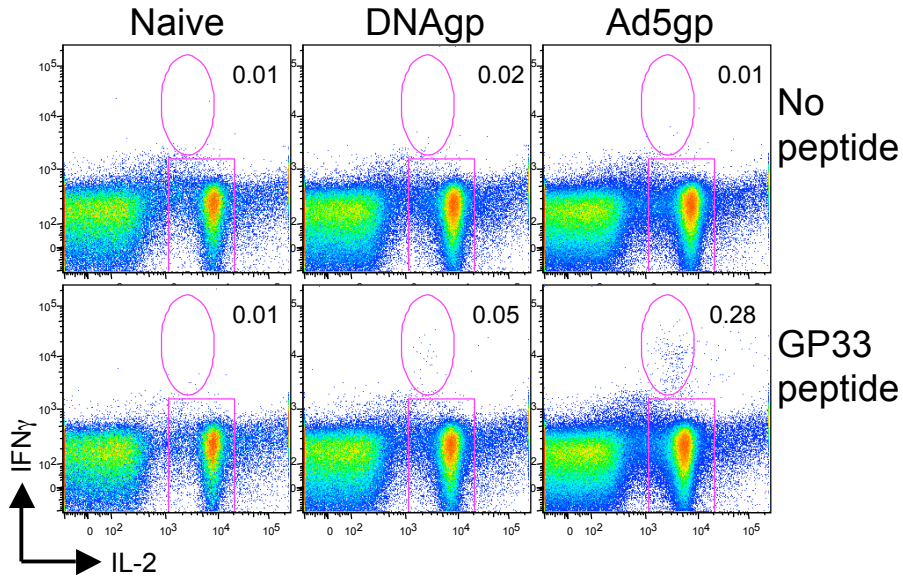
A. Representative intracellular cytokine staining of splenocytes

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. Naïve=non-immunized B6 mouse. Percentages are %IFNγ⁺ of CD8 T cells



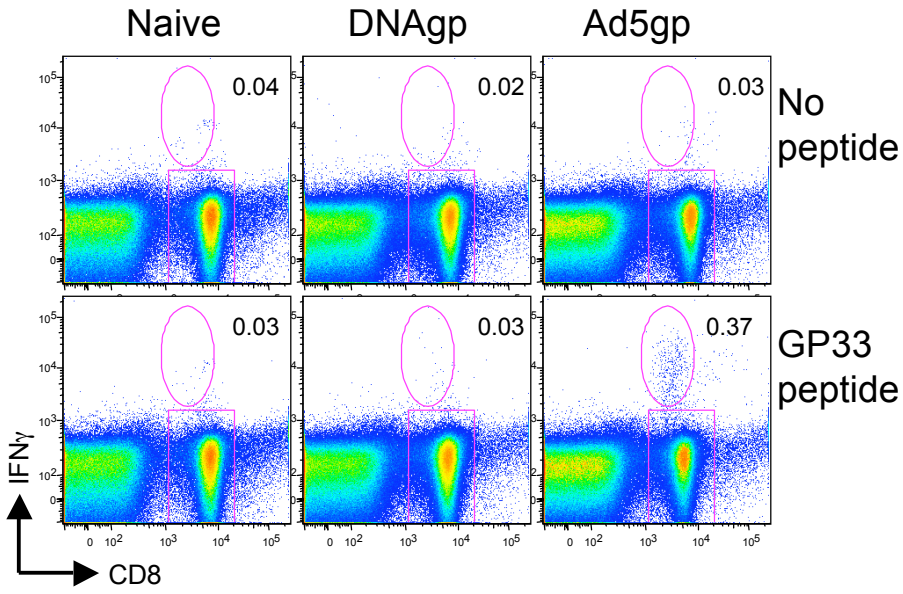
B. Representative intracellular cytokine staining of inguinal lymph node cells

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. Naïve=non-immunized B6 mouse. Percentages are %IFNγ⁺ of CD8 T cells



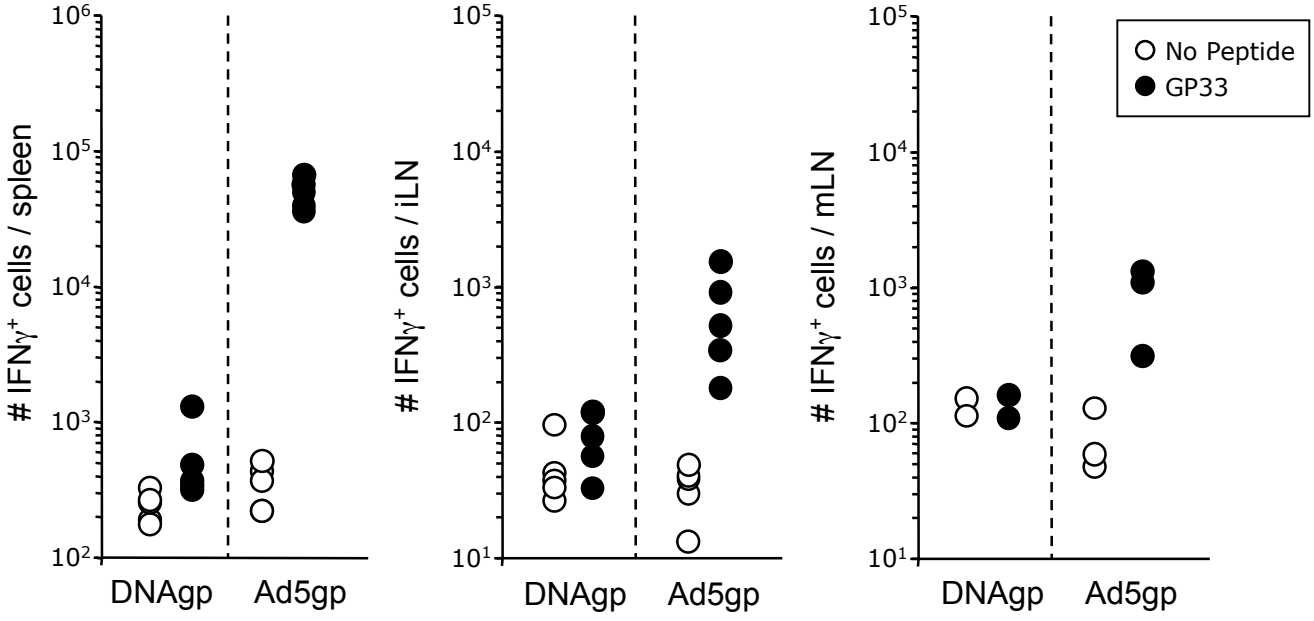
C. Representative intracellular cytokine staining of mesenteric lymph node cells

Single cell suspensions of mesenteric lymph nodes 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. Naïve=non-immunized B6 mouse. Percentages are %IFNγ⁺ of CD8 T cells.



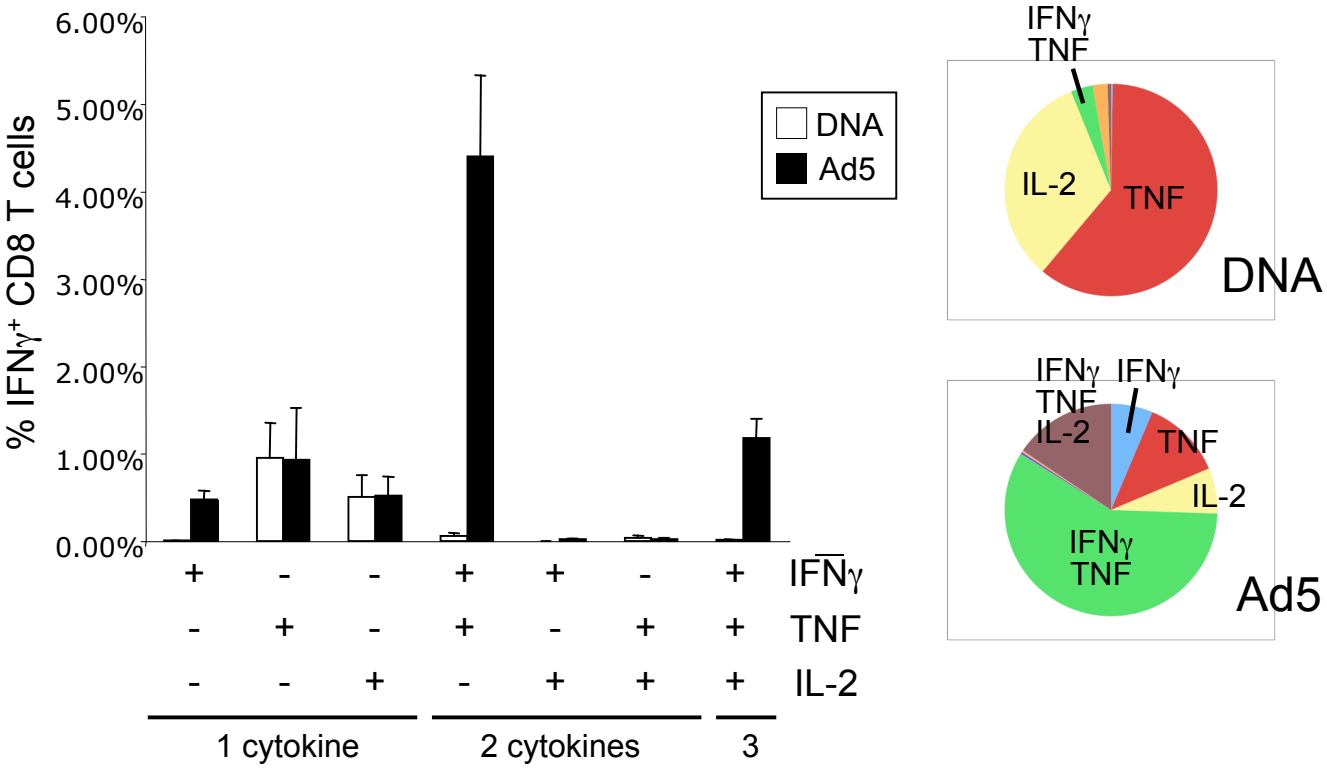
D. Quantitation of GP33-specific CD8 T cells by ICS

Number of IFNγ⁺ cells is derived from cell recovery and % CD8⁺IFNγ⁺ data from ICS. iLN=inguinal LN, mLN=mesenteric LN.



E. Analysis of polyfunctional CD8 T cells by intracellular cytokine staining.

Single cell suspensions of splenocytes were 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,TNF, and IL-2. Percentages are of CD8 T cells. Pie graphs show percentage of total cells producing each cytokine or combinations of cytokines.



Summary cytokine analysis:

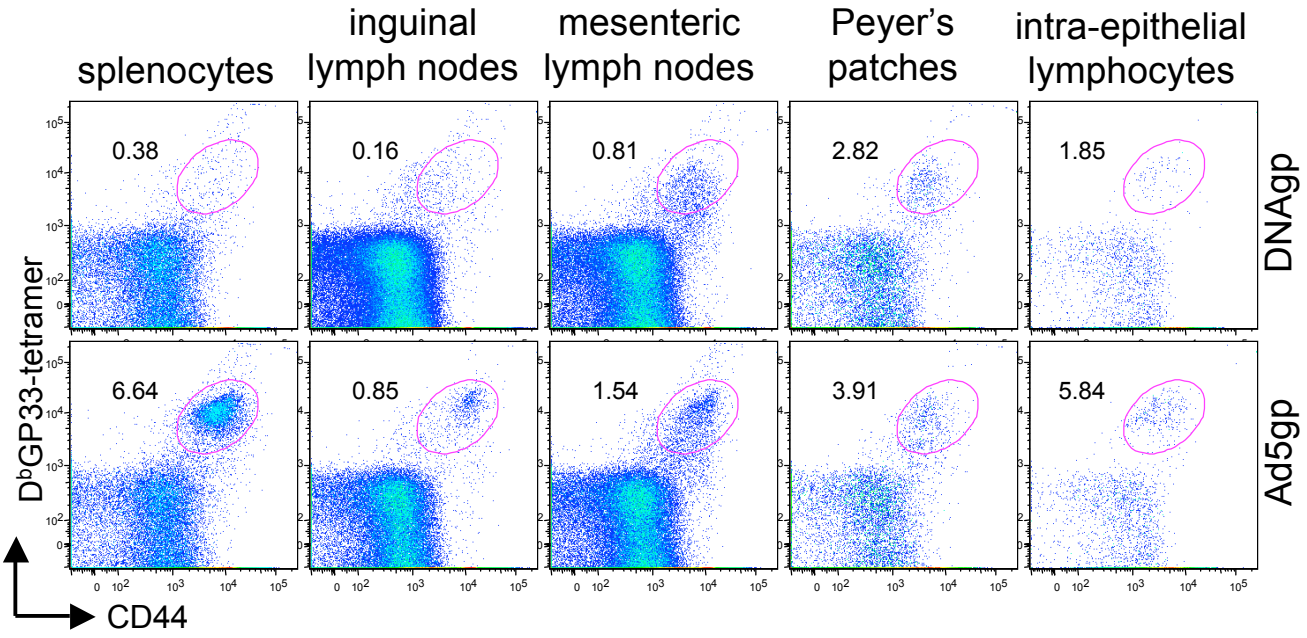
Overall memory T cell frequencies were higher in Ad5 immunized mice, but memory cells were still detectable in spleen and inguinal lymph nodes of DNA immunized mice. The responses elicited in DNA tended to produce only IL-2 or TNF, with few cells producing IFNγ and TNF. This was dramatically different than responses in Ad5 immunized mice which tended to have cells producing multiple cytokines. This suggests that the vaccine-elicited memory CD8 T cells in DNA immunized mice may have a more "naïve" phenotype as most of these cells do not produce IFNγ. The % of cells producing IL-2 or TNF only was similar between DNA and Ad5 immunized groups, despite large differences in the fraction of cells producing IFNγ only or combinations of cytokines.

II. MHC tetramer staining:

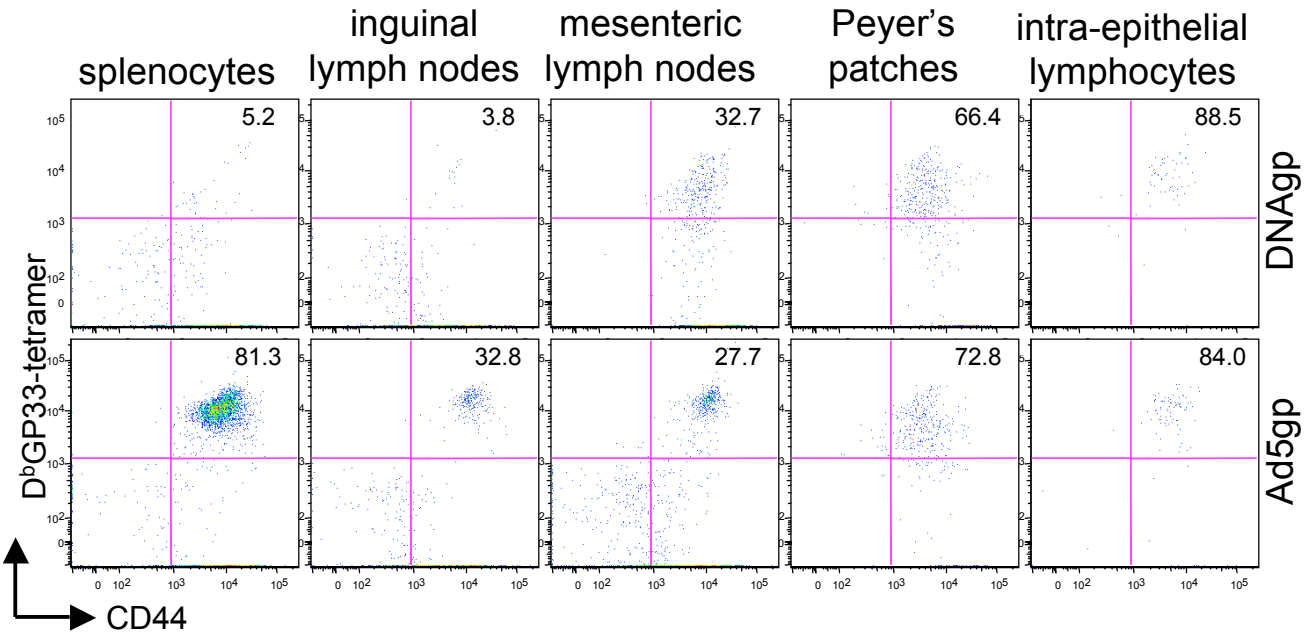
A. Representative tetramer staining of cells from immunized mice

Single cell suspensions from the indicated tissues of immunized mice at 30 days post DNAgp or 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.

Gated on total $CD8^+$ T cells



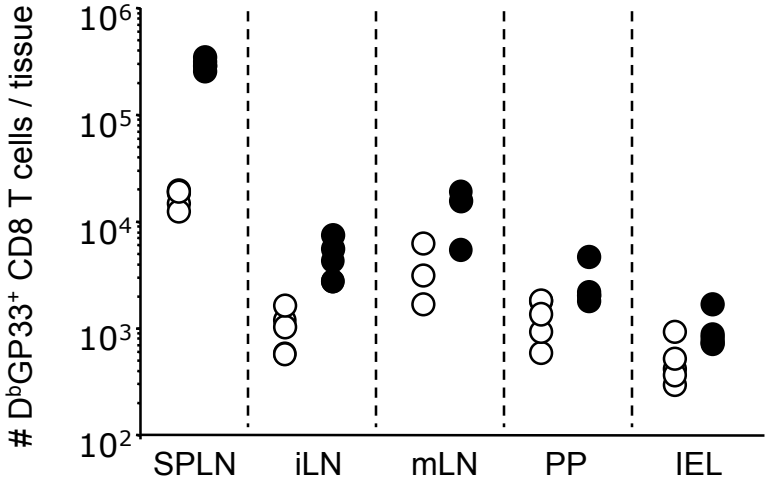
Gated on donor Thy1.1$^+$ $CD8^+$ T cells



II. MHC tetramer staining:

B. Quantitation of GP33-specific T cells by tetramer staining

Numbers of GP33-specific CD8 T cells calculated from FACS plots. SPLN=spleen, iLN=inguinal lymph node, mLN=mesenteric lymph node, PP=Peyer's patches, IEL=gut intraepithelial lymphocytes.

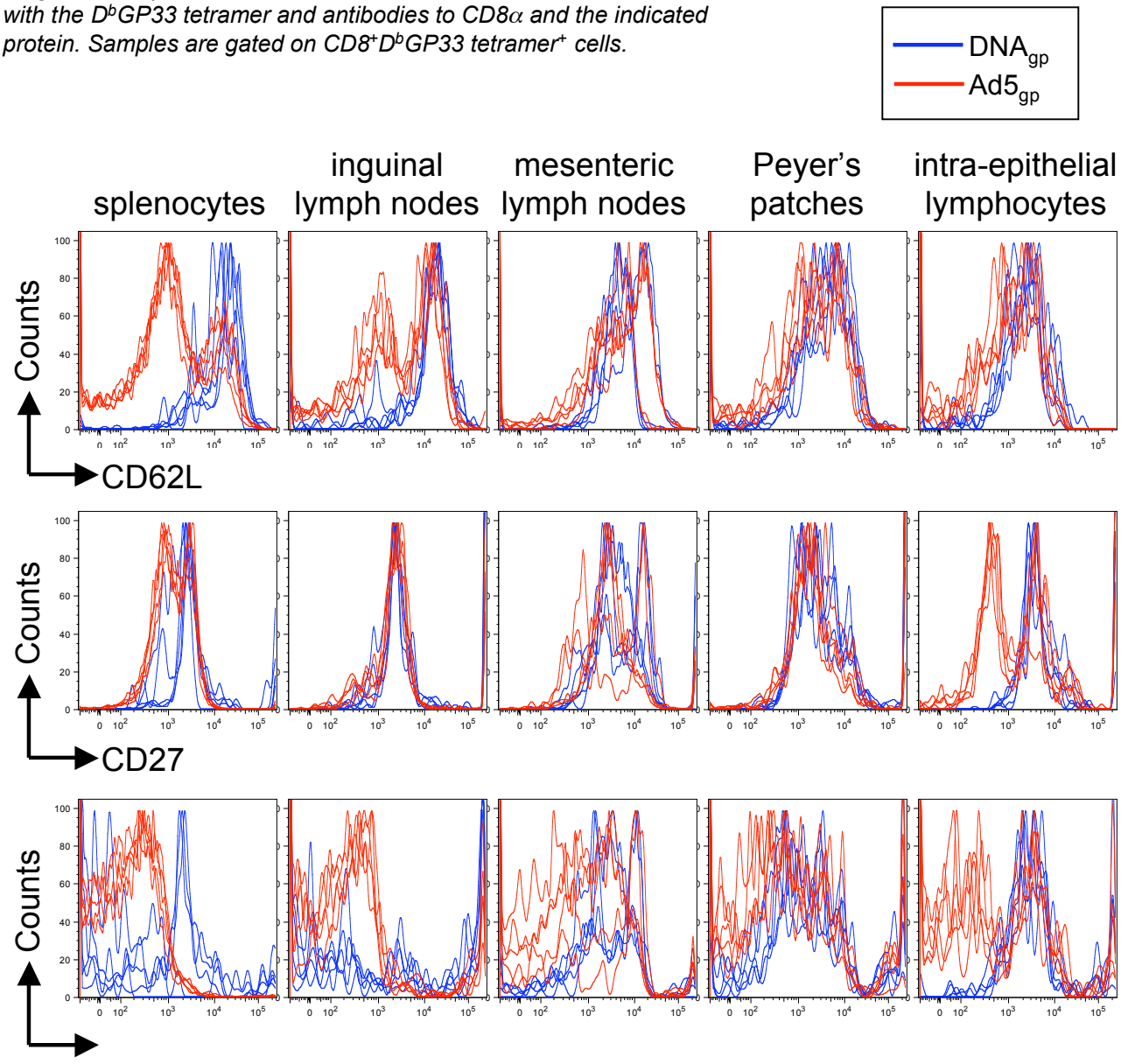


Summary Tetramer analysis:

Similar to results obtained from intracellular cytokine analysis, overall memory T cell frequencies were higher in Ad5 immunized mice compared to DNA immunized mice. This was true either when looking at total responses or only donor responses in immunized mice. However, the large difference in memory T cell frequencies observed in the spleen was less in other sites, with similar numbers of vaccine-induced antigen specific CD8 T cells in some mucosal sites such as the mesenteric lymph nodes, peyer's patches, and gut epithelia. Consistent with analysis of peak responses, DNA immunized mice had responses in mucosal sites that were not represented in systemic sites such as the spleen.

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.



Summary phenotype analysis:

Although overall memory T cell frequencies 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. This is consistent with data from intracellular cytokine staining in which the detected cells were skewed towards those that were able to produce IL-2 suggesting that these cells had a naïve or "central-memory" phenotype. Conversely, The majority of cells from Ad5 immunized mice were CD62L^{lo} which was also consistent with the observation that the majority of these cells were unable to produce IL-2 suggestive of a "effector-memory" phenotype. Also consistent with these results, A higher proportion of memory T cells in DNA immunized mice were CD27$^+$ and CD127$^+$. However, the actual numbers of cells from Ad5 and DNA primed mice that were CD62L^{hi}, CD27$^+$, and CD127$^+$ was similar.