frequency of V_H genes derived from the HIV Env-reactive blood and colostrum B cells. However, V_H gene subfamily 1 ~ 69 usage was more frequent among colostrum-derived (51.4%) than blood-derived (20.4%) HIV Env-reactive antibodies ($p = 0.005$, Fisher's exact test). In addition, colostrum contained a higher percentage of gp120-directed antibodies and a lower percentage of gp41-directed antibodies than blood (65.7 and 14.3% gp120-specific, and 34.3 and 77.6% gp41-specific, respectively; $p < 0.0001$, Fisher's exact test). One cross-compartment HIV Env-specific clonal B cell lineage was identified.
Conclusion: The IgG1 isotype predominance, more restricted V_H gene usage, and higher percentage of gp120-specificity of HIV Env-specific antibodies isolated from colostrum B cells compared to peripheral B cells of HIV-1-infected women suggest selective homing of restricted populations of IgG-secreting memory B cells to the lactating mammary gland. Thus, effective maternal vaccination to eliminate postnatal virus transmission may require specific targeting of this distinct population of mucosal B cells.

P03.77 LB
Vaccine-Elicited B Cell Responses Against the HIV-1 Primary Receptor Binding Site

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Background: Due to the high level of structural variability of HIV-1 Env, elicitation of neutralizing antibody (NAb) responses to conserved neutralizing determinants, such as the CD4 binding site (CD4bs), is a major focus of HIV-1 vaccine development. Accordingly, a detailed understanding of how trimeric Env immunogens activate the naïve primate B cell repertoire and how Env-elicited antibody responses evolve during the course of the immunization schedule may indicate how to direct the response toward desired neutralizing targets.
Methods: Recently, we established methodology to characterize NAb responses elicited by soluble Env immunogens in rhesus macaques by antigen/epitope-specific single B cell sorting, RT-PCR to recover immunoglobulin G (IgG) heavy/light chain genes, in vitro expression of IgG, and epitope fine-mapping to the conserved CD4bs. Here, we extend the use of this methodology to conduct a comprehensive analysis of the evolving vaccine-elicited CD4bs-specific B cell response at the clonal level.
Results: We interrogated the Ab repertoires from two rhesus macaques following a total of five inoculations with trimeric Env. We observed similar VH gene usage in the CD4bs- and Env-specific memory B cell repertoires while the level of somatic hypermutation was higher following the 5th immunization compared to following the 2nd immunization in both compartments. Interestingly, the heavy chain CDR3 regions from the CD4bs-specific B cells were on average longer than those from the total Env-specific cells and the CD4bs-specific B cell subset has higher level of clonality than that of the Env-specific subset. However, only a small portion of the CD4bs-specific Ig repertoire following the 2nd immunization is maintained following the 5th immunization, which suggests that clonality is only partially maintained and the repertoire is dynamically recruiting new B cells to generate diversity.
Conclusion: These data provide an improved understanding of the evolving B cell response following Env immunization.

P03.78 LB
Computational Analysis of Anti-HIV-1 Antibody Neutralization Panel Data to Identify Potential Functional Epitope Residues

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Background: Advances in single cell antibody cloning methods have led to the identification of a variety of broadly neutralizing anti-HIV-1 antibodies. Initial characterization of these antibodies often involves measurement of their neutralization activity against a panel of viruses, but such experiments do not generally lead to conclusive identification of an antibody's epitope.
Methods: We developed a computational tool, Antibody Database, to help identify critical residues on Env whose natural variation affects antibody activity. Our simplifying assumption was that for a given antibody, a significant portion of the dispersion of neutralization activity across a panel of HIV-1 strains is due to the amino acid identity or glycosylation state at a small number of specific sites, each acting independently. A model of an antibody's neutralization IC50 was developed in which each site contributes a term to the logarithm of the modeled IC50. The analysis program attempts to determine the set of rules that minimizes the sum of the residuals between observed and modeled IC50s.
Results: As a test case, we analyzed antibody 8ANC195, an anti-gp120 antibody of unknown specificity. The model for this antibody indicated that glycosylation sites at Env positions 234 and 276 were critical for neutralization. We evaluated this prediction by measuring neutralization potencies of 8ANC195 against HIV-1 in vitro and in an antibody therapy experiment in humanized mice.
Conclusion: These experiments confirmed that 8ANC195 represents a distinct class of glycan-dependent anti-HIV-1 antibody and validated the utility of computational analysis of neutralization panel data. The Antibody Database program implements this analysis as well as providing an environment where sequence, neutralization, and structural data can be examined together.

P04.34 LB
Immunogenicity of MVA-B in HIV-1-Infected Volunteers

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Background: Previous studies suggested that poxvirus-based vaccine regimens may be instrumental in the therapeutic HIV field. Here, we have characterized the T cell mediated immunity elicited in 22 chronic HIV-1-infected patients undergoing highly active antiretroviral therapy (HAART) pre- and post-vaccination with a clade B-based HIV-1 vaccine candidate expressing Env, Gag, Pol and Nef antigens (MVA-B).
Methods: A total of 30 chronic HIV-1-infected patients on HAART with CD4 cell counts above 450 cells/mm3 and undetectable viremia were enrolled in a phase I, double-blind, placebo-controlled trial. The volunteers were randomly allocated to receive three injections of MVA-B (108 PFU/ dose) ($n = 20$) or placebo ($n = 10$) by intramuscular route at weeks 0, 4 and 16