Robust early expansion of rare HIV bnAb precursors is crucial to drive the development of cross-neutralizing serum responses

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Despite extensive efforts, an effective HIV vaccine remains elusive. One promising approach is germline-targeting immunogen design. We developed the germline-targeting immunogen Q23-APEX-GT2, which successfully primed long-CDRH3 V2-apex bnAb precursors in knock-in mice and elicited focused responses in rhesus macaques. In macaques, Q23-APEX-GT2 consistently induced V2-apex bnAb precursors, but serum neutralization was variable across six vaccinated animals. Notably, one animal, CH35, exhibited weak but detectable heterologous neutralization at week 14 following a homologous prime (week 0) and boost (week 10). To investigate this variability, we performed longitudinal antigen/epitope-specific B cell profiling using 10X Genomics single-cell sequencing and bulk NGS, tracing antibody/B cell lineages. CH35 displayed greater lineage diversity and early robust clonal expansion relative to other animals. Flow cytometry of lymph node (LN) and peripheral blood mononuclear cells (PBMCs) further revealed that CH35 had a higher frequency of antigen-specific memory B cells in PBMCs, which strongly correlated with serum neutralization. These findings suggest that LN- and PBMC-derived B cells, potentially seeding into bone marrow, are key contributors to serum neutralizing antibody titers. To test whether primed lineages could mature into bnAbs, animals were challenged at week 18 with a SHIV encoding the CAP256.SU Env, following Q23-APEX-GT2 prime–boost immunization. Post-infection, we isolated monoclonal antibodies from CH35, several of which traced back to Q23-APEX-GT2–primed lineages. At least two lineages demonstrated expanded neutralization breadth, with one lineage exhibiting the broadest activity. Together, these results provide compelling evidence that early robust expansion of rare HIV bnAb precursors through germline-targeting immunogens can seed cross-neutralizing serum antibody responses. This study offers critical insights for advancing HIV vaccine design, particularly in refining efficient germline-targeting priming strategies.