Naïve B cells From Unvaccinated Rhesus Macaques Cross-React With HIV gp140 Protein

Michelle Premazzi Papa[1], Andrew Wilson[1], Rosemarie Mason[2], Jennifer Manuzak[3], Nichole Klatt[4], Rebecca Lynch[1]

[1] Dept. of Microbiology, Immunology, and Tropical Medicine, GWU, Washington, DC, USA, [2] Vaccine Research Center, NIH, Bethesda, MD, USA, [3] Division of Immunology, Tulane National Primate Research Center; New Orleans, LA, USA, [4] Department of Surgery, University of Minnesota, Minneapolis, MN, USA.

THE GEORGE WASHINGTON UNIVERSITY
WASHINGTON, DC

CROI Conference on Retroviruses and Opportunistic Infections

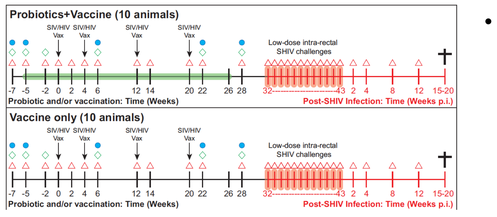
0384

BACKGROUND

HIV causes a chronic infection that it is not cleared by the normal immune response, in part due to an ineffective early antibody response[1]. Other groups have shown that early in infection, people with HIV-1 (PWH) develop anti-gp41 antibodies that cross-react with commensal bacteria but do not neutralize HIV[2], and that this same phenomenon occurs after vaccination[3]. Here, we investigate if the baseline antibody response prior to HIV vaccination is comprised of antibodies targeting commensal bacterial that weakly target HIV, and if these antibodies are preferentially activated after subsequent infection.

METHODS

A) Samples from a previous vaccine study



Klatt 2021 NPJ Vaccines

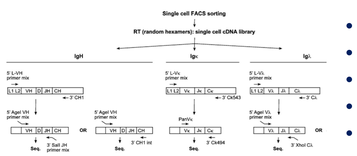
- PBMC samples from a SIV/HIV DNA vaccine (SIV gag (p55), HIV env (gp160), and gp140 trimeric protein) and simian-human immunodeficiency virus (SHIV) challenge study in Rhesus Macaques (RhM; n=8) were used.
- Vaccination in the presence and absence of probiotics.
- Challenged with heterologous SHIV.
- Selected sequences were cloned as IgG1 and tested for function.

B) B cell staining and sorting

Specificity	*Fluor*	*Functionality*
Viability	Aqua Blue	Viability Stain
CD3	PerCP-Cy5.5	Exclusion gate
CD8	BV711	Exclusion gate
CD4	BV785	Exclusion gate
CD14	PE-Cy7	Exclusion gate
CD20	BV605	B cells marker
IgD	FITC	Igs to detect class switched cells
IgM	BV650	Igs to detect class switched cells
IgG	PE-Cy5	Igs to detect class switched cells
gp140 Probe I	APC	To mark antigen specific B cells
gp140 Probe II	PE	To mark antigen specific B cells

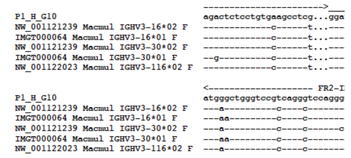
C) BCR Gene Analysis

Amplifying and sequencing



- IgG
- IgM
- IgA
- Kappa
- Lambda

Analyzing Genetically



D) Gating strategy for B cell sorting

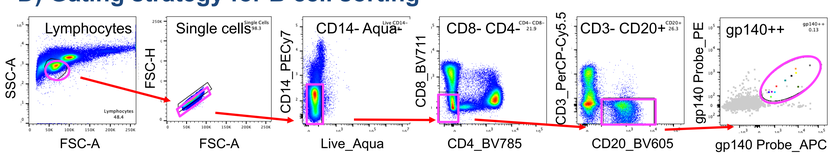
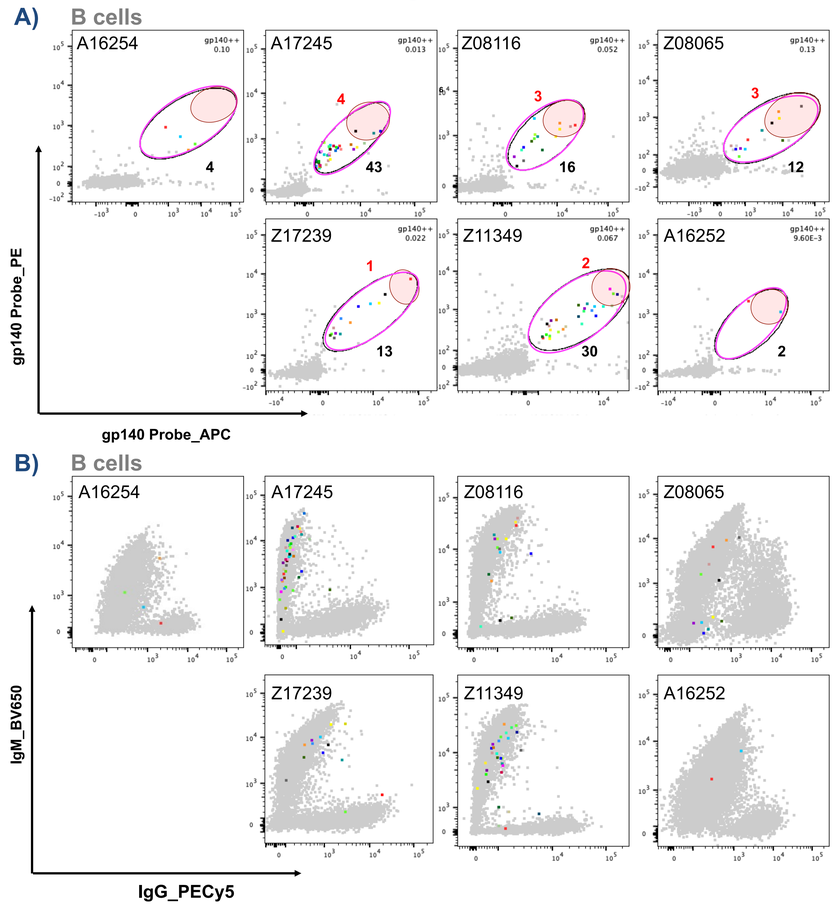


Figure 1: In collaboration with Dr Klatt, we received PBMCs from a previous vaccine study in Rhesus Macaques[4]. Samples were collected at week -7 (before any treatment) (A). PBMCs were stained using a 11-color B cell panel and antigen-specific B cells were sorted (B). Nested-PCR was performed using specific primers for IgG, IgM, IgA heavy chains and Kappa and Lambda light chains. Samples were sent for sequencing and the best sequences had the Germline analyzed using IMGT/V-QUEST (C). Representative gating strategy for B cell sorting (D).

RESULTS

B cells from unvaccinated rhesus macaques bind to HIV gp140



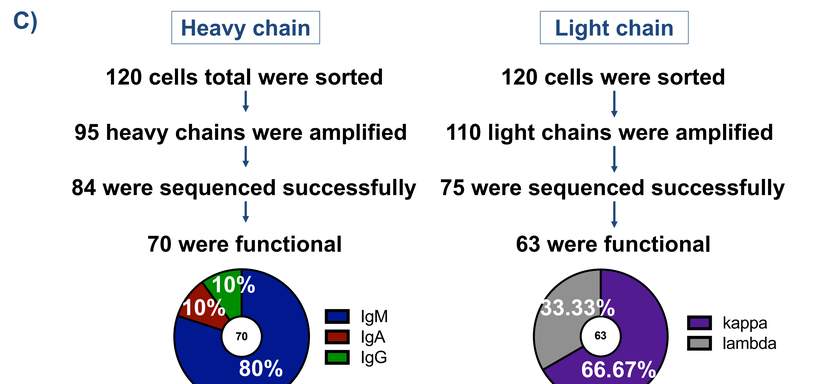


Figure 2: B cells double positive for gp140 protein were sorted from 7 rhesus macaques. Colored dots indicate sorted B cells for each animal. A) **Black** numbers indicate cells sorted and **red** indicate cells with high binding to gp140 chosen for cloning. B) IgM and IgG analysis of B cells (in grey) and sorted cells (colored) for each animal. C) Summary of heavy and light chains analyzed using IMGT/V-QUEST. Majority of pre-existing gp140-binding cells are circulating naïve B cells.

RESULTS

mAbs bind to HIV proteins but do not neutralize HIV

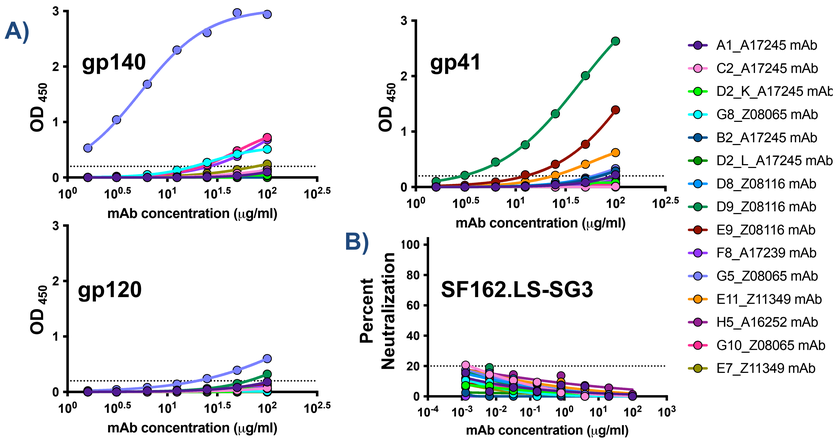


Figure 3: A) ELISA plates were coated gp140, gp41 and gp120 HIV proteins and incubated with serially diluted mAbs. B) mAbs were serially diluted and incubated with SF162.LS-SG3 and percent of neutralization was measured. Dotted lines indicate the limit of detection of the assay. mAbs can recognize HIV although low levels of binding were observed. As expected, these mAbs do not neutralize HIV.

mAbs cross-react with bacterial and human antigens

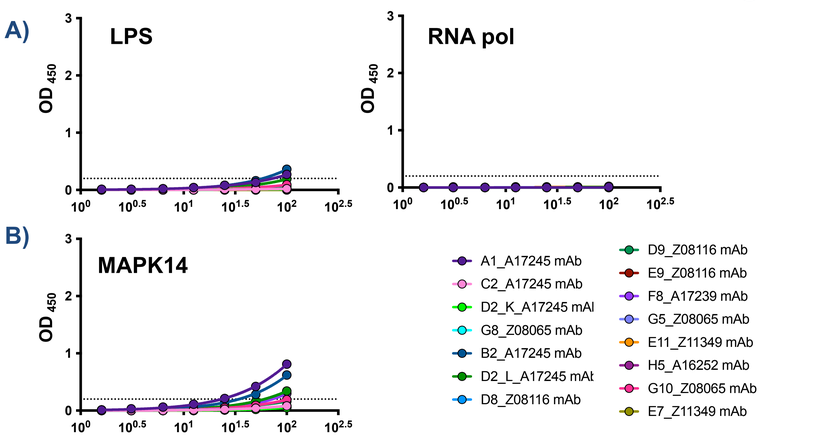


Figure 4: ELISA plates were coated with bacterial antigens LPS and RNA polymerase (A), or human MAPK14 (B) and incubated with serially diluted mAbs. Dotted lines indicate the limit of detection of the assay. Two mAbs bound to LPS and MAPK14 indicating that these might be polyreactive antibodies.

CONCLUSIONS

- Majority of pre-existing gp140-binding cells are circulating naïve B cells.
- mAbs cloned from naïve B cells can recognize HIV proteins albeit with low levels of binding. More mAbs recognize gp41 than gp120.
- Two mAbs recognize bacterial and human antigens suggesting that these might be polyreactive antibodies.

REFERENCES

1. Tomaras GD, Haynes BF. HIV-1-specific antibody responses during acute and chronic HIV-1 infection. Curr Opin HIV AIDS. 2009 Sep;4(5):373-9. doi: 10.1097/COH.0b013e32832f00c0. PMID: 20048700; PMCID: PMC3133462.
2. Trama AM, *et al*., HIV-1 envelope gp41 antibodies can originate from terminal ileum B cells that share cross-reactivity with commensal bacteria. Cell Host Microbe. 2014 Aug 13;16(2):215-226. doi: 10.1016/j.chom.2014.07.003. PMID: 25121750; PMCID: PMC4294419.
3. Williams, *et al*., (2015). HIV-1 VACCINES. Diversion of HIV-1 vaccine-induced immunity by gp41-microbiota cross-reactive antibodies. Science (New York, N.Y.), 349(6249), aab1253.
4. Klatt NR, *et al*., Effects of persistent modulation of intestinal microbiota on SIV/HIV vaccination in rhesus macaques. NPJ Vaccines. 2021 Mar 11;6(1):34. doi: 10.1038/s41541-021-00298-4. PMID: 33707443; PMCID: PMC7952719.