NASCENT HIV-1 ENV-REACTIVE bNAb INDUCTION IN SHIV-INFECTED NEONATAL RHESUS MACAQUES

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BACKGROUND

- Recent studies have indicated that HIV-1 envelope (Env)-reactive broadly neutralizing antibodies (bnAbs) are generated more frequently and within a year of infection in infants and children[1,2].
- Understanding the cellular and molecular mechanisms of infant bnAb development will inform strategies for early-life HIV immunization that can generate bnAbs.

PURPOSE OF STUDY

To develop a neonatal model for heterologous HIV-1 NAb induction that may inform immunogen design to elicit bnAbs by future pediatric HIV-1 vaccines

METHODS

- 13 neonatal RMs (therapy-naïve) were intravenously infected with simian-HIV (SHIV) CH848 10.17 DT.E169K post-birth (Fig. 1).
- Plasma Viral Load was monitored by quantification of SIV p27 (RNA copies/ml)[3].
- Single genome amplification of HIV-1 envelopes (N=1212) in plasma from longitudinal timepoints[3].
- Plasma Abs were tested for binding to synthetic peptides, recombinant HIV-1 Env gp120 and stabilized chimeric SOSIP trimers in ELISA[4].
- Plasma Abs from all RMs over time were tested for neutralization of autologous and heterologous HIV-1 references strains via TZM-bl assay[5].
- Germinal center B and T cell phenotyping using previously described markers via flow cytometry[6].
- Transcriptomic profile of PBMCs from SHIV-infected neonatal RMs. Gene libraries were generated using the 5' library construction kit and processed on 10X Chromium Connect (10X Genomics)[4].

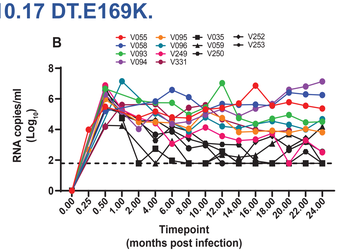
RESULTS

Figure 1. Study design and viral dynamics neonatal RMs infected with SHIV CH848 10.17 DT.E169K.

A

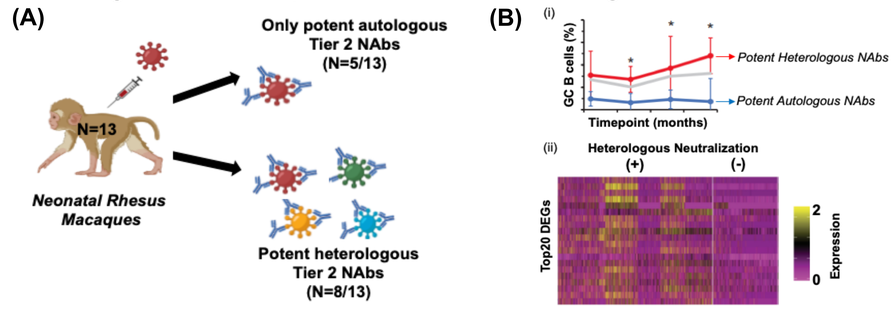
Animal ID	Sex	Mamu alleles			Infection timepoint	Virus Inoculum (ng)
		A*01	B*08	B*17		
V094	Male	-	-	+	Day 4	5
V096	Male	-	-	+	Day 4	50
V253	Male	-	-	+	Day 4	50
V331	Male	+	-	-	Day 4	50
V250	Male	-	-	-	Week 4	50
V058	Male	-	-	-	Week 4	50
V035	Male	-	-	+	Week 7	5
V055	Male	-	-	+	Week 7	50
V252	Female	-	-	+	Day 4	50
V095	Female	-	-	-	Week 3	50
V059	Female	-	-	-	Week 4	50
V249	Female	-	-	+	Week 4	50
V093	Female	-	-	-	Week 6	50

B



(A) Information on the cohort of neonatal rhesus macaques (RMs) studied including their sex, genotype for SIV restrictive *Mamu*-alleles and viral inoculum used to establish infection. **(B)** Viral load dynamics. SIV gag RNA was measured via qPCR to assess plasma viral loads and was reported as RNA copies/ml (Log_{10}). ***Neonatal RMs were successfully infected with SHIV CH848 10.17 DT.E169K and demonstrated viral kinetics similar to human HIV-1 infection.***

Hora *et al.* develop a neonatal rhesus macaque model for induction of polyclonal neutralizing antibodies (NAbs) against heterologous tier 2 HIV-1 strains following pathogenic simian-HIV (SHIV) infection. Understanding the mechanisms of neonatal heterologous HIV-1 NAb induction will provide insights into future pediatric HIV-1 vaccine designs.

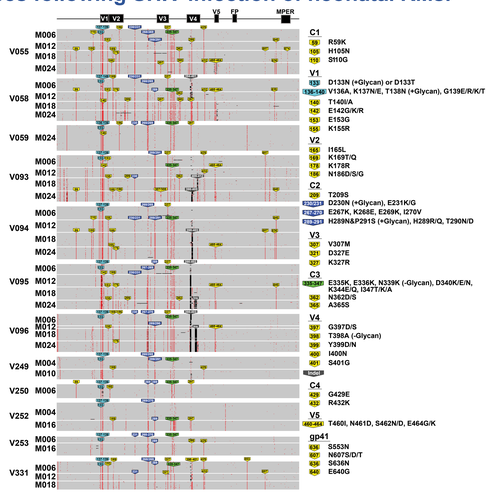


(A) Thirteen neonate rhesus macaques infected with a pathogenic SHIV generated autologous and heterologous tier 2 or difficult-to-neutralize HIV-1 strains. All 13 RMs generated autologous tier NAbs, but a subset (8/13) also developed heterologous NAbs with increasing potency overtime.
(B) To define the immunological factors associated with heterologous neutralization in neonate RMs, we studied the frequency of germinal center B and T cells (i) as well as the transcriptome signature of peripheral blood cells (ii) in RMs that generated potent heterologous NAbs compared with those who only generated potent autologous NAbs.

We developed a neonatal RM model of heterologous tier 2 NAb induction, which provides a key resource to study neonatal bnAb induction that may inform future design of pediatric HIV-1 vaccines.

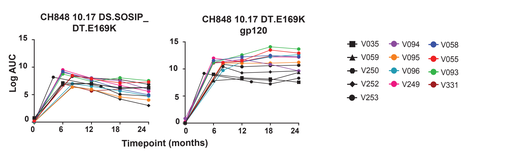
RESULTS Contd.

Figure 2. Mutational patterns in HIV-1 Env NAb-epitopes following SHIV infection of neonatal RMs.



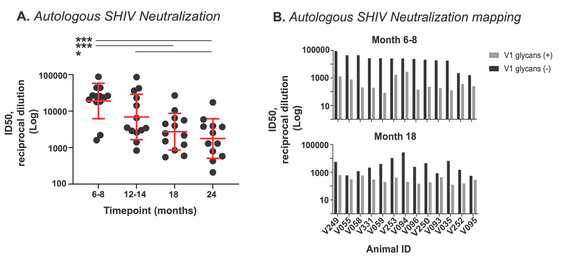
Pixel plot visualizing the mutations (red lines) that emerged over time in the autologous *envs* following infection with SHIV CH848 10.17 DT.E169K *env* in neonate RMs. Envelope sequences were generated via SGA. ***Env evolution in SHIV CH848 10.17 DT.E169K infected neonate RMs was associated with selection pressures at multiple sites, including those targeted by bnAbs.***

Figure 3. Binding profile of plasma antibodies elicited by SHIV infection in neonatal RMs



SHIV infection in neonatal RMs elicited a polyclonal antibody response in ELISA. ***Plasma from all RMs revealed binding Abs to recombinant HIV-1 Envs.***

Figure 4. Neutralization profile of Autologous NAbs elicited by SHIV infection in neonatal RMs.



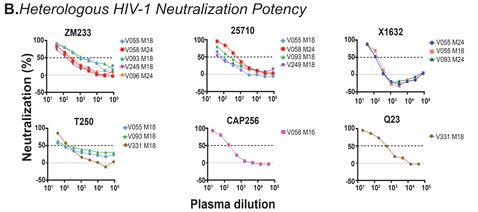
Autologous plasma HIV-1 NAbs were generated in all 13 RMs with neutralization titers significantly decreasing over time from month 6 to 24. Plasma at 6-8 and 18 months post SHIV infection in all RMs were tested for neutralization of autologous SHIV CH848 10.17 DT.E169K (-V1 glycans), and CH848 10.17 E169K (+V1 glycans) in TZM-bl cells. ***Pathogenic SHIV infection in neonate RMs elicited potent autologous tier 2 NAbs that wane overtime, suggestive of viral escape to evade host immunity.***

Figure 5. Neutralization profile of Heterologous NAbs elicited by SHIV infection in neonatal RMs.

A. *Heterologous HIV-1 Neutralization Breadth*

Neutralization breadth; heterologous HIV-1 strains											
Animal ID	V058	V093	V331	V055	V096	V095	V094	V249	V253	V250	V252
HIV-1 neutralization	4/8 (50%)	4/9 (44%)	2/5 (40%)	2/6 (33%)	2/6 (33%)	2/7 (29%)	1/6 (17%)	1/7 (14%)	0/5 (0%)	0/5 (0%)	0/6 (0%)

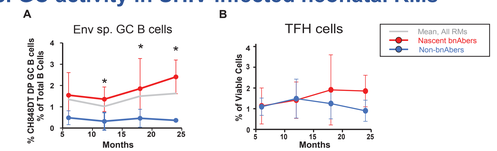
B. *Heterologous HIV-1 Neutralization Potency*



Development of heterologous neutralizing plasma antibodies post-infection. **(A)** Percentage of the viruses neutralized by longitudinal samples per animal with increasing neutralizing titers overtime. **(B)** Neutralization curves of plasma from representative RMs with maximal neutralization titers against heterologous tier 2 HIV-1 strains. ***Pathogenic SHIV infection in neonate RMs elicited potent heterologous tier 2 NAbs, suggestive of bnAb induction.***

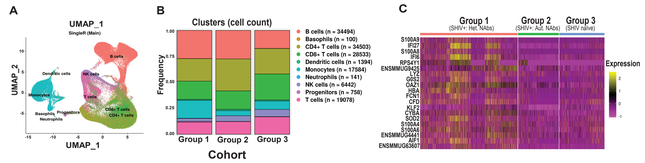
RESULTS Contd.

Figure 6. GC activity in SHIV-infected neonatal RMs



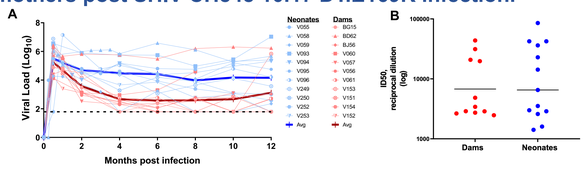
GC activity in SHIV-infected neonatal RMs. **(A)** Frequencies of Env-specific GC B cells in neonate RM bnAbers *versus* non-bnAbers (blue); the gray line shows the average of all animals. Frequencies of TFH cells in RM bnAbers (red) *versus* non-bnAbers (blue). ***Nascent bnAbers have robust on-going GC responses following SHIV infection.***

Figure 7. Transcriptomics of peripheral blood cells in SHIV-infected neonatal RMs.



(A) Immune cell subsets inferred by SingleR based on transcriptome sequencing. **(B)** Frequency of cell subsets in RMs from three groups; Group 1: heterologous neutralization (SHIV+); Group 2; autologous neutralization (SHIV +); and Group 3; SHIV-naïve. **(C)** Heatmap showing the top 20 differentially-expressed genes (DEGs) that overlapped between the three groups. ***RMs generating heterologous HIV-1 NAbs have more activated immune cell subsets.***

Figure 8. Comparison of viral dynamics and autologous neutralization titers in the neonatal RMs with their dam rearing mothers post SHIV CH848 10.17 DT.E169K infection.



Plasma viral load titers and autologous neutralizing antibodies in neonatal RMs and their respective dam rearing mothers. **(A)** Longitudinal viral load titers over the course of 12 months measured by SIV gag RNA via qPCR and reported as RNA copies/ml (Log10). Average viral loads are represented by thick lines. **(B)** Plasma neutralization titers measured as ID50 at month 12 following infection. Virus tested: autologous SHIV CH848 10.17.DT.E169K. ***Neonates and adults have different viral dynamics following pathogenic SHIV infection, but generated similar levels of autologous NAbs.***

CONCLUSIONS

- Pathogenic SHIV infection of neonatal RMs elicited autologous and heterologous tier 2 NAbs, suggestive of bnAb induction.
- HIV-1 Envs within SHIV infected neonatal RMs evolved with selection pressures in sites targeted by NAbs, including bnAbs.
- Development of plasma heterologous neutralization in neonate RMs was associated with more activated immune cells in blood and enhanced germinal center activity.
- Pathogenic SHIV replicated better in neonate RMs, but both mom and babies generated similar levels of autologous NAbs suggestive of different mechanisms of NAb induction in neonates and adults.

ADDITIONAL KEY INFORMATION

References

[1]Goo et al., Nat. Med., 2014, [2]Muenchhoff et al., Sci. Tranls. Med., 2016, [3]Li et al., PNAS, 2016, [4]Williams et al., Cell, 2021, [5]decamp et al., J. Virol., 2014, [6]Havenar-Daughton et al., Cell Rep., 2019

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