Dynamic Changes of B Cell Lineages during cART in Chronic SHIV-infected Juvenile Rhesus Macaques

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Introduction

- HIV-1 infection alters the frequencies and functions of B cell subsets, which may be restored by combined antiretroviral therapy (cART).[1]
- A threshold of virus replication (~60 days) may be required to induce autologous neutralizing antibodies (ANAbs), after which they continue to expand on suppressive ART to better target the range of reservoir variants.[2]
- There is still a gap in our understanding of the maturation of autologous and heterologous HIV-1 NAbs (NAbs), including those that may develop into broadly NAb status, during cART initiation.
- Host immunity after cART initiation in pediatric populations living with HIV (LWH) is understudied.

Previous Observations

- We recently established a macaque model of neonatal Simian-HIV (SHIV)-infection that generated autologous and heterologous HIV-1 NAbs with characteristics of bNAbs over time in young rhesus macaques (RMs).[3,4]
- We now have the opportunity to evaluate the dynamics of B cell lineages with autologous and/or heterologous HIV-1 neutralization activities during cART initiation in chronic SHIV-infected juvenile RMs.

Purpose of Study

To interrogate the dynamics of B cell clones pre- and post- cART in a chronic SHIV infection model of monkeys.

Methods

- We studied 9 male and female juvenile RMs that were all *Mamu-A*01*-negative and infected 4 days to 7 weeks post birth (Table 1).[3,4]
- The 9 juvenile RMs were intravenously infected with SHIV CH848 10.17 N133DN138T (DT).E169K as infants for ~2-4 years in the absence of therapy, followed by 2 years of daily cART that included a single treatment interruption event at 12 months after cART initiation in 6/9 RMs (Figure 1). Three of 9 RMs spontaneously controlled viremia and remained therapy-naïve.
- Peripheral blood cells (PBMCs) and tissue (lymph node) samples were collected over time post-infection.
- Plasma viral load was quantified via qPCR of SIV gag RNA copies/mL.[3,4]
- Pre-cART, plasma Abs were tested for autologous and heterologous HIV-1 neutralization activities in TZM-bl cells.[3-6]
- Post-cART, purified IgG derived from plasma samples were tested for autologous SHIV neutralization in TZM-bl cells.[2]
- Post-cART, purified IgGs were epitope-mapped structurally onto autologous HIV-1 envelope (Env) trimer via electron-microscopy-based polyclonal epitope mapping (EMPEM).[7]
- We used the 10X Genomics single cell immune profiling assay to study the B cell receptors (BCRs) and transcriptomes of single B cells that were negatively enriched from longitudinal PBMCs, pre and post cART.[3,5]
- Antigen-specific B cell phenotyping in longitudinal PBMCs, pre and post cART, via flow cytometry; data not shown.[3,5]
- Quantification of intact proviral DNA in purified CD4+ T cells derived from PBMCs.[8]

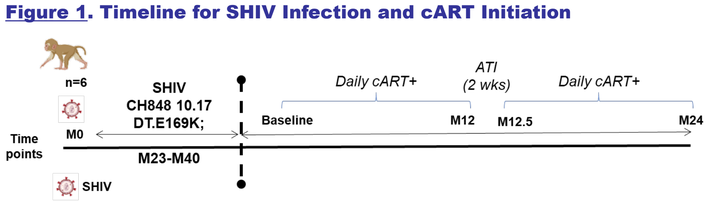
Results

Table 1. Juvenile Macaque SHIV Infection

Animal ID	Sex	*Mamu*-typing A*01	*Mamu*-typing B*08	*Mamu*-typing B*17	Age at time of infection	Virus inoculum (ng)	cART initiation/ Age range
V059	F	-	-	-	Week 4	50	Daily cART: M40-M66
V095	F	-	-	-	Week 3	50	Daily cART: M39-M65
V096	M	-	-	+	Day 4	50	Daily cART: M39-M65
V249	F	-	-	+	Week 4	50	Daily cART: M30-M56
V253	M	-	-	+	Day 4	50	Daily cART: M31-M57
V331	M	+	-	-	Day 4	50	Daily cART: M23-M49
V035	M	-	-	+	Week 7	5	Therapy-naive
V250	M	-	-	-	Week 4	50	Therapy-naive
V252	F	-	-	+	Day 4	50	Therapy-naive

Shown are sex, genotype for SIV restrictive *Mamu*-alleles [negative (-) and positive (+)], and viral inoculum (ng P27) used to establish infection at the indicated age; days/ weeks in infant RMs. Age at time of cART initiation shown in months (m).

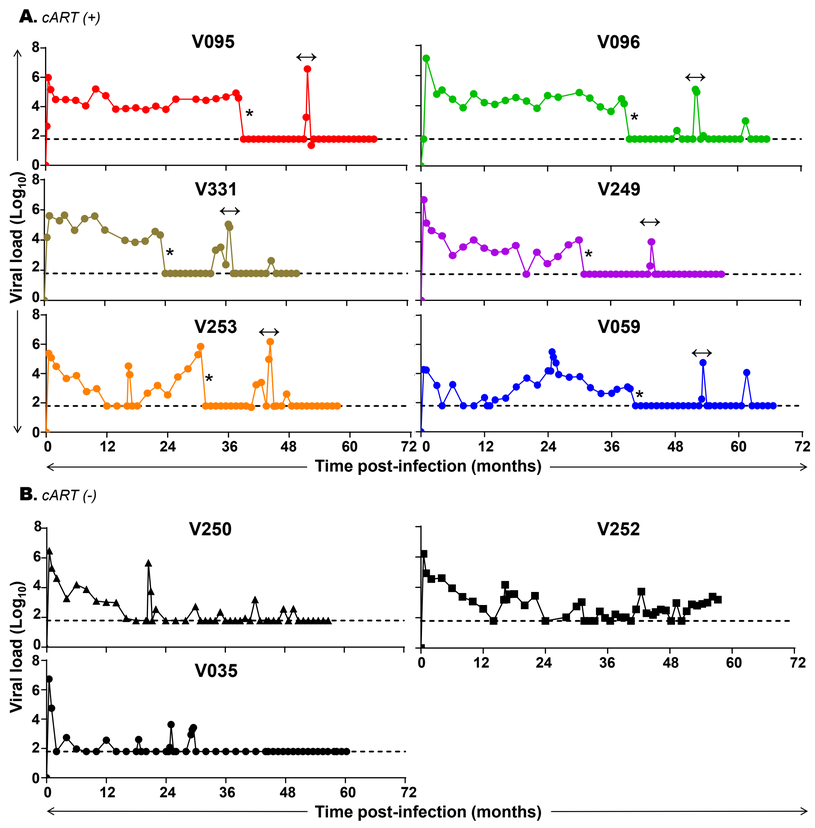
Figure 1. Timeline for SHIV Infection and cART Initiation



Overview of therapy-naïve neonatal SHIV infection followed by cART initiation in a subset of RMs (N=6). The remaining 3 of 9 RMs (Table 1) remained therapy naïve following SHIV infection. There was an cART treatment interruption (ATI) event for two weeks due to unavailability of cART. RMs were infected with a SHIV encoding CH848 10.17 N133DN138T (DT).E169K that that was associated with heterologous HIV-1 NAb induction in young RMs, prior to cART initiation. [3,4]

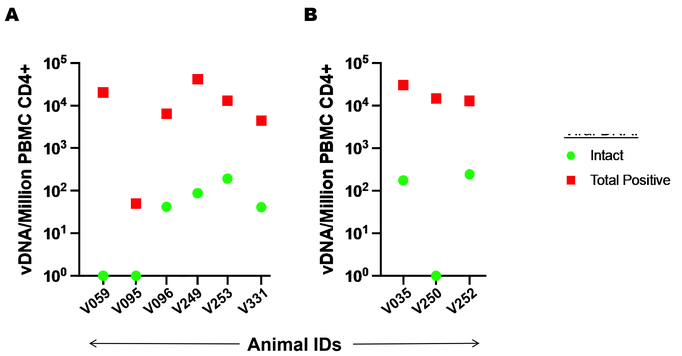
Results (continued)

Figure 2. Viral Dynamics: Only RMs with persistent viremia were cART-treated, and RMs that spontaneously controlled viremia remained cART-naïve.



Plasma viral load was measured as SIV gag RNA copies/mL following SHIV infection of neonate RMs that were either treated with daily cART during months 23-66 post infection **(A)** or remained therapy-naïve **(B)**. Symbols (panel A): *cART initiation (see also Table 1); ↔ ATI for 2 weeks. The limit of detection is indicated by the horizontal dash line. **In general, daily cART treatment suppressed viremia to low or undetectable levels in juvenile RMs during chronic SHIV infection.**

Figure 3. Virus Reservoirs: Chronic SHIV-infected juvenile RMs had a range of virus reservoir with the intact around 100 per million CD4+ T cells.



The size of the viral reservoirs in purified PBMC-derived CD4+ T cells was measured via IPDA in chronic SHIV-infected RMs that were treated with cART **(A)** or remained therapy-naïve **(B)**. Shown are the viral DNA copies per million CD4+ T cells in each sample; intact DNA (green) and total DNA (red). Timepoint studied was month 9 post cART initiation or the equivalent timepoint in therapy-naïve RMs. **Chronic SHIV-infected juvenile RMs had detectable intact virus reservoirs.**

Figure 4. HIV-1 Neutralization Profile Pre-cART: Young RMs had autologous and/or heterologous HIV-1 plasma NAbs during chronic SHIV infection.

Neutralization titer, ID50: <20 | 20-100 | 100-1000 | 1000-10,000 | >10,000

Animal group	Animal ID	Pre-cART timepoint (MPI)	Neutralization titers, ID50/ Heterologous tier 2 HIV-1: SHIV CH848 10.17 DT.E169K	246-F3	25710	BG505/ T332N	BJOX	CAP256	Ce1176	CH119	CNE55	Q23.17	T250-4	TRO.11	X1632	X2278	ZM233	MuLV
G1; cART+ (M23-66 MPI)	V059	M36	7423	<20	<20	<20	<20	<20	<20	<20	<20	<20	32	<20	<20	<20	21	<20
	V095	M36	2251	<20	35	<20	<20	<20	21	<20	20	<20	32	<20	35	<20	67	<20
	V096	M36	3272	<20	39	<20	<20	36	<20	<20	<20	<20	31	<20	<20	<20	161	<20
	V249	M30	1268	<20	35	<20	<20	<20	<20	<20	<20	<20	29	<20	28	<20	32	<20
	V253	M30	2390	107	315	181	254	339	270	226	115	543	648	102	136	263	465	81
	V331	M24	1307	131	136	213	165	212	224	131	163	1017	512	81	139	283	148	69
G2; cART-	V035	M36	10,195	<20	<20	<20	<20	<20	<20	<20	<20	<20	24	<20	<20	<20	<20	<20
	V250	M30	2008	<20	<20	<20	<20	<20	<20	<20	<20	<20	32	<20	<20	<20	<20	<20
	V252	M30	3385	<20	22	<20	<20	32	23	<20	<20	<20	72	<20	<20	<20	65	<20

Plasma Abs from one of the last timepoints in chronic SHIV-infected RMs prior to cART treatment at 23-66 months post-infection (MPI) in Group 1 RMs, and matching timepoints post-infection in Group 2 RMs that remained therapy-naïve, were tested for neutralization of autologous and heterologous HIV strains as described [3,4,6]. Neutralization titer was measured in TZM-bl cells and reported as ID50 (reciprocal dilution); as shown by the key, the heatmap was color coded to show the magnitude of NAb titers. Positive neutralization titer was set at ≥3X over the negative control virus (MuLV). **Plasma NAbs had heterologous HIV-1 neutralization, characteristics of bNAbs, albeit of limited potency.**

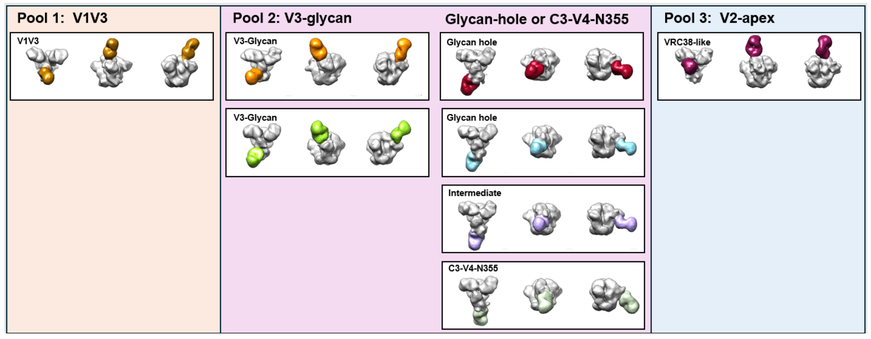
Figure 5. Autologous HIV-1 Neutralization Activity Post-cART: Purified IgG in young RMs after cART initiation in chronic SHIV infection maintained neutralization activity.

IC50, µg/ml: 0.1-1 | 1-10 | 10-100 | 100-1000 | >1000

Virus ID	Neutralization titers (IC50, µg/ml) of purified IgG (M9/equiv. post cART): cART (+) V059	V095	V096	V249	V253	V331	cART (-) V035	V250	V252
SHIV CH848 10.17 DT.E169K	7.9	32	231	16	12	36	3.8	6.4	44

Purified IgG from plasma of chronic SHIV-infected RMs at month 9 post-cART or equivalent timepoint in RMs that remained therapy-naïve were tested for neutralization of autologous SHIV in the TZM-bl assay. Neutralization titer was reported as IC50 (µg/ml); the heatmap was color coded to show the magnitude of NAb titers. Positivity cutoff for neutralization was IC50 = 1000µg/ml. **Plasma HIV-1 neutralization activity is maintained after cART treatment.**

Figure 6. Polyclonal IgG Epitope Specificities: Chronic SHIV infection elicited IgGs of varied Env-binding specificities, including those targeting bNAb epitopes.

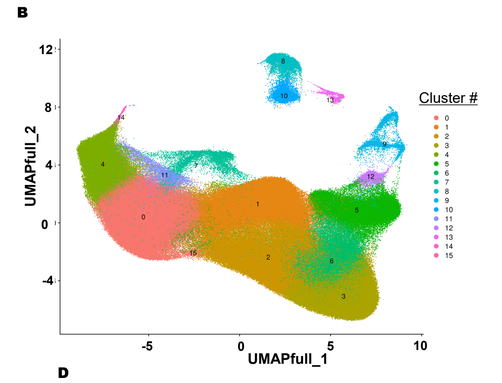


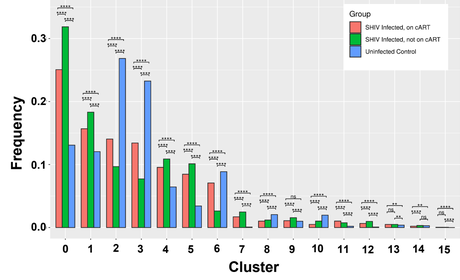
Plasma-derived purified IgG from chronic SHIV-infected juvenile RMs were structurally analyzed via EMPEM. Purified IgG were digested into Fabs (~1mg) and complexed with 20 mg CH848 DS DT E169K SOSIP overnight at 4°C. Each complex was purified by SEC and concentrated prior to being subjected to routine negative stain electron microscopy (NSEM). NSEM data was collected on an EM420 electron microscope and images were analyzed and 3D classifications and final refinements were calculated with routine protocols using Relion 3.0[9]. *Shown are binding specificities for Fabs each group of animals during chronic SHIV infection in the absence of therapy (months (M) 20-40 post-infection); **Pool 1** (V035 M40, V059 M37, V250 M26); **Pool 2** (V249 M26, V253 M26) and **Pool 3** (V095 M36, V252 M28 and V331 M20). Samples were pooled based on purified IgG neutralization titers during chronic SHIV infection (see Fig. 5).*

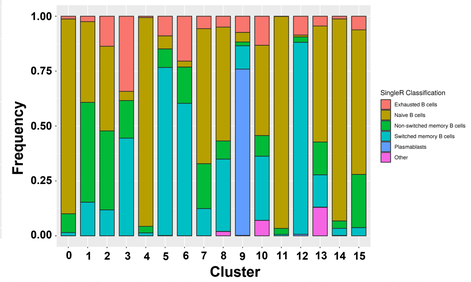
Figure 7. Transcriptomics of B cells in Chronic SHIV Infection (+/- cART)

A

Group	Monkey ID	Timepoint Post cART	B cells/ GEX	B cells/ Fxnl 1:1 VH+VL
SHIV Naïve	DE09	n/a	61101	21615
	DG60	n/a	50858	15884
	DH89	n/a	46909	11034
SHIV Infected; cART (+)/ 23-66 months post-infection	V249	M0	80883	7484
		M1	51397	6042
		M12	54846	6170
	V096	M0	8635	3155
		M1	16590	5754
		M12	7172	2323
	V059	M0	25882	9341
		M6	11934	4952
		M12	4095	1174
	V253	Pre-cART	47326	20910
		M2	24764	3143
		M13	44520	20039
	V331	Pre-cART	20845	6269
		M7	25830	5069
		M13	43918	17974
	V095	M0	50948	8521
		M1	32551	8731
		M13	31072	13022
SHIV infected; cART (-)	V250	M0 equiv.	64372	13703
		M12 equiv.	56797	10634
	V252	M0 equiv.	52366	12405
		M12 equiv.	15277	3136
	V035	M0 equiv.	30483	9115
		M13 equiv.	48293	22366

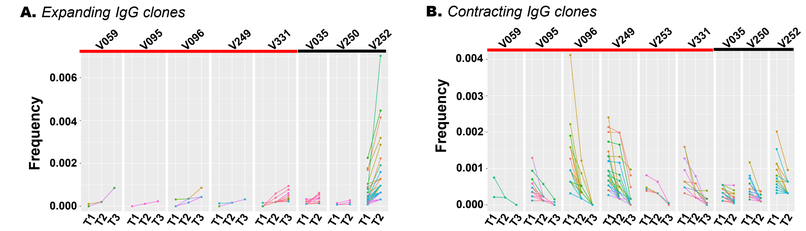






Negatively-enriched single B cells from PBMCs in RMs were studied via 10X Genomics single immune cell profiling assay; SHIV-naïve young RMs (31-43 months old) and SHIV-naïve juvenile RMs that were either treated with cART (M23-66 months post-infection) or remained therapy-naïve. For therapy-naïve SHIV-infected RMs, we studied equivalent timepoints relative to duration of infection compared to the animals that were cART treated. **(A)** Animals studied and timepoints relative to cART or infection as mentioned above. Number of B cells from which we recovered transcripts for gene expression (GEX) analysis as well as BCRs with paired heavy (VH) and light (VL) chain genes (1:1). **(B)** Unique clusters of B cells; each dot represents a single cell and cells cluster based on similar GEX profiles. **(C)** Frequency of B cells from each group of animal (key in panel insert) that constitutes each cluster. Fisher's exact test and Bonferroni p-value adjustment in R. **** is adjusted p-value < .0001. **(D)** Frequency of B cells from different predicted B cell subsets in each cluster. B cell subsets were inferred computationally using monkey gene reference databases in SingleR as described[5]. **Transcriptomics of B cells after cART initiation in chronic SHIV infection revealed subsets with predicted memory phenotypes suggestive of cART-restored functional memory B cells in infection; for example, clusters 2, 3 and 6 that contained predominantly cells from SHIV-infected RMs on cART and SHIV-naïve RMs, compared to therapy-naïve SHIV-infected RMs.**

Figure 8. Dynamics of B cell Clones after cART Initiation in Chronic SHIV Infection



Frequency of B cell clonal lineages that either expanded **(A)** or contracted **(B)** during chronic SHIV infection in RMs. Lines beneath animal IDs: red – cART(+); black – cART(-). Timepoint – T1 (M0 or pre-cART), T2 (M1/6/7) and T3 (M12/13); see also Fig. 6A. Expansion was defined as increase in clonal lineage members from T1-T3; the reverse is true for contraction. B cell clonality was computationally inferred via Cloanalyst[5]. **Expansion and contraction in B cell clone sizes reflects the dynamics of B cells during cART initiation in chronic SHIV infection.**

Summary

- Pre-ART plasma or IgG in SHIV infection have functional and/or structural characteristics of b/NAbs.
- Post-ART IgG autologous neutralization activities of varied potencies are indicative of NAb persistence during cART.
- Computationally-inferred clonally-related BCRs in SHIV-infected RMs (+/- cART), including strictly expanding or contracting IgG clones across longitudinal timepoints.
- Transcriptomics revealed memory phenotype B cells during cART initiation in chronic SHIV infection of juvenile RMs that may support ART-restored functional memory B cell subsets in infection.

Conclusions and Relevance for HIV Cure

- B cell lineage persistence/ expansion underlies their maturation during cART that may translate to a similar process in PLWH that may be driven by residual viremia within germinal centers.
- cART initiation in pediatric populations LWH will support B cell development that can also be harnessed via therapeutic HIV-1 vaccines.

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References: [1]Goonetilleke *et al.* Front Immunol. 2019; [2]Whitehill *et al.* JCI. 2024; [3]Hora *et al.* Cell Rep. 2023; [4]Holmes et al. Nat Comm. 2024; [5]Williams *et al.* Cell. 2021; [6]deCamp *et al.* J Virol. 2014; [7]Saunders *et al.* Cell. 2024; [8]Abdel-Mohsen *et al.* Nat Med. 2020; [9]Zivanov *et al.* eLife. 2018.