Multivariant HIV-1 infection in infants is associated with the induction of broadly neutralizing plasma antibodies.

Nitesh Mishra[1#], Shaifali Sharma[1#], Ayushman Dobhal[1#], Sanjeev Kumar[1], Ravinder Singh[2], Bimal Kumar Das[2], Rakesh Lodha[3], Sushil Kumar Kabra[3], Kalpana Luthra[1*].

[1]Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India – 110029.

[2]Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India – 110029.

[3]Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, India – 110029.

[#]These authors contributed equally to this work.

*Correspondence – Kalpana Luthra (kalpanaluthra@gmail.com)

Abstract

Due to the extensive antigenic diversity of HIV-1, broadly neutralizing antibodies (bnAbs) develop in a subset of infected individuals over 2-3 years of infection[1,2]. Interestingly, infected infants have been shown to develop plasma bnAbs frequently and as early as one-year post-infection[3], with features atypical than adult bnAbs[4,5], suggesting the factors governing bnAb induction in infants are different than those in adults. Understanding the antigenic features in infants with early bnAb responses will provide key information on the antigenic triggers driving B cell maturation pathways towards induction of bnAbs. Herein, we evaluated the presence of plasma bnAbs in HIV-1 clade C perinatally infected infants of Indian origin and identified the viral factors associated with early bnAb responses. A strong association of multivariant infection in infant elite neutralizers with development of plasma nAbs targeting diverse autologous viruses was observed. Our data provides information supportive of polyvalent vaccination approaches for HIV-1.

Introduction, Results and Discussion

In order to assess HIV-1 specific plasma bnAb breadth in our cohort of 51 infants, we first evaluated nonspecific inhibitory effect by assessing the inhibition of MuLV infection in a TZM-bl based pseudovirus neutralization assay. Of the 51 plasma samples, 4 showed nonspecific inhibitory effect and were excluded from further analysis. To identify infants with early bnAb responses, we next performed plasma neutralization activity (PNA) of the remaining 47 infants against a panel of 20 genetically divergent pseudoviruses[6–8], with 13 viruses representing global viral diversity and 7

additional viruses of Indian origin (**Supplementary Table 1**), in order to capture plasma bnAbs targeting diversity encountered in the context of Indian HIV-1 pandemic (**Figure 1A and Supplementary Figure 1**). Overall, 19/47 (42%) infants showed cross-clade neutralization activity, signified by the ability to neutralize non-clade C pseudoviruses (different clade than the infecting clade) at ID50 titers >50, at a median time of 12-months post-infection (p.i.) (range = 6-24 months). Though the PNA in infants was relatively broad with 26% (12/47) of infants neutralizing >50% of the pseudoviruses, their potency in comparison to PNA from chronically infected children and adults showed relatively lower magnitude (**Figure 1B and C**), which prompted us to define pediatric elite neutralizers (ENs) and broad neutralizers (BNs) in the context of a modified breadth-potency matrix. The normalized neutralization scores, predictive of geometric mean titres and cross-clade neutralization, were calculated **(Figure 1D – E and Supplementary Figure 2**). A cut-off of 0.7 defined the 90th-percentile boundary and was used to define ENs (neutralizing ≥90% pseudoviruses), whereas a cut-off of 0.35 (75th-percentile) was used to define BNs (neutralizing ≥60% pseudoviruses) (**Table 1**). The neutralization scores for known ENs and BNs from previously reported pediatric[9] and adult[10] cohorts were calculated based on the above criteria, confirming the validity of the panel and the scoring system defined herein to decipher pediatric broad and elite neutralizers (**Figure 1E**).

Using the HIV-25710-2.43 mutant pseudoviruses, we next delineated the epitope specificities of plasma bnAbs from ENs and BNs. The plasma bnAbs of majority of the infants (8/11) were directed against the V2-glycan, with two ENs (AIIMS704 and AIIMS706) showing multi-epitope dependency, a feature reported to be typically associated with chronic antigenic exposure[8,11] (**Figure 1F**). Despite the high variability in terms of the sequence, glycosylation and length of the V2-apex of HIV-1 envelope, bnAbs directed against V2-apex are elicited relatively early and are one of the most potent classes of bnAbs[1,12]. The findings herein of a high frequency of V2-apex bnAbs in infants with PNA is in consensus with previous observations in infected children[11]. In AIIMS704, dependence on V2-apex and MPER were observed while AIIMS706 showed dependence on V2-apex and V3-glycan (**Figure 1F**). Antibodies against MPER are rarely elicited in children, plausibly due to the structural constraints in accessing the MPER as well as autoreactivity of MPER bnAbs. Our findings of MPER nAbs in a 12-month old infant (AIIMS704) suggests similar bnAbs can be induced early with targeted vaccination strategies that can plausibly overcome the linked immune tolerance mechanisms blocking such nAbs.

We next focused our analysis on four parameters (three viral: viral load, CD4 count and duration of infection and one patient-related: gender) to identify factors driving bnAb induction. No significant association was observed between CD4 counts or duration of infection with neutralization breadth (**Figure 2A and B**), suggesting these parameters did not influence bnAb induction in this cohort of infants. High viral load emerged as a negative driver of neutralization breadth ($r = -0.497$, $p = 0.002$) (**Figure 2C**), implicating a possible antigenic saturation in our cohort. Alternatively, it is plausible that infants with plasma bnAbs were better able to control viral replication. However, since all infants were initiated on antiretrovirals after testing HIV-1 positive, longitudinal assessments could not be performed. A significant difference was observed between male and female cross neutralizers (odd ratios >3, $p = 0.04$), with the frequency of bnAb inducers higher in case of males than females (**Figure 2D – F**).

Another factor influencing bnAb induction is the diversity in the viral envelope glycoprotein (env). Diversity in env, either generated by immune selection pressure (neutralization escape) or superinfection, has been shown to be an independent driver of bnAb induction[8,13–15]. To define viral diversity in the context of bnAb induction, we performed SGA analysis of env sequences[16] (V1C5 region of HIV-1 gp120) from circulating viral variants in ENs and BNs to assess the impact of viral diversity on bnAb induction. From each sample, more than 30 env sequences were generated, giving a 90% confidence interval of sequencing variants present at 10% population frequency. Maximum likelihood phylogeny and pairwise raw distance distribution were assessed to identify the pattern of viral transmission and intra-host diversity. Of the four ENs, three showed multivariant HIV-1 infection (two distinct branches on phylogeny tree[17]), with one infant, AIIMS709, showing two highly divergent variants, plausibly due to superinfection, that needs to be further explored (**Figure 3A, Supplementary Figure 3 – 4**). The SGA env sequences from BNs were monophyletic, with long branch lengths in some cases, suggestive of highly diverse infecting viruses (**Figure 3A**). In addition, high evolutionary divergence was observed in ENs compared to BNs ($p = 0.006$) (**Figure 3B – C, Supplementary Table 2**). Moreover, the multivariant infection in this infant cohort was significantly associated (odds ratio >35, $p = 0.043$) with development of plasma breadth, though a small sample size might have skewed the association. HIV-1 multivariant infection is defined as one person infected with two or more different HIV-1 strains. According to the timing of infection with the second strain, multivariant infections can be divided into co-infection[18] (acquisition of a second strain either

simultaneously or before seroconversion) and superinfection[19,20] (acquisition of a second strain after seroconversion), but given the cross-sectional nature of this study, we could only categorize the multivariant infections as co-infection.

Multivariant HIV-1 infection is more commonly seen in adults than in children who have acquired the infection by vertical transmission[16,17,21,22]. The stringent genetic bottleneck for transmission in perinatally infected infants often leads to infection by a single viral variant[23]. To gain insight and evaluate the impact of multivariant infection in development of plasma nAbs, we next generated functional pseudoviruses from all four ENs. The generated pseudoviruses were tested for neutralization by autologous nAbs. Distinct viral populations in each of the infected infant showed varied susceptibility to autologous plasma nAbs, with several variant sensitive and others moderately sensitive to autologous plasma bnAbs (**Figure 3D**), an observation we previously reported to be the driver of elite plasma neutralization activity[8]. Pseudoviruses were then probed for their susceptibility to known bnAbs, and non-nAbs to assess their antigenic profile and potential as candidate components for epitope-targeted HIV-1 vaccine. Majority of the pseudoviruses showed similar neutralization susceptibility profile to V2-apex targeting bnAbs while the susceptibility to other bnAb classes varied between pseudoviruses. No reactivity was observed with non-nAbs for majority of the pseudoviruses, suggesting a well-ordered trimeric configuration (**Figure 3D**). In addition, notable sequence similarity was observed in the strand B and C of the V2 loop (**Figure 3E**). V2-germline-priming immunogens are directly based on native viral variant (native-like trimers) configuration[24,25], providing the feasibility to engineer templates with relative ease compared to other bnAb germline targeting epitopes. Therefore, viral variants from these ENs can be explored as priming immunogens to induce HIV-1 V2-apex specific neutralizing responses, though their ability to induce inferred germlines prototypes of V2-apex bnAbs still remains to be explored.

In this cross-sectional study on a small cohort of HIV-1 clade C infected infants, we have demonstrated a strong association of multivariant infection with the development of plasma bnAb response. Though exposure to two distinct viral variants in adults has been shown to be not sufficient to broaden neutralizing antibody responses[26–28], our findings of an early bnAb response in context of multivariant infection can be a distinct feature of infant immune response to HIV-1 infection. This needs to be validated in established cohorts of HIV-1 infected infants. Our data provides key evidence for exploring polyvalent vaccination approaches for pediatric HIV-1 infection. Polyvalent vaccines

have been less explored due to immunodominance of HIV-1 which in turn can diminish the efficacy of vaccines[29,30]. We observed the plasma nAbs in infants with multivariant infection to target both variants, suggesting env specific antibodies generated in context of two distinct viral variants can target epitopes on both envelopes. In addition, viral variants from these infants can be explored as candidate priming immunogens to elicit V2-apex targeting bnAbs. Given the relatively higher frequency, earlier induction, moderate level of somatic hypermutation, and consistent cross-clade neutralizing activity of V2-apex bnAbs in conjunction with cross-species conservation of the epitope given its critical function for trimer disassembly during viral entry[31–35], this bnAb epitope has been focus of recent immunogen design approaches. Furthermore, longitudinal analysis in established infant cohorts should be undertaken to understand how the immune system in infants responds to multiple HIV-1 strains which will provide key insights for guiding the early development of such bnAbs.

Materials and Methods

Study Population

All infants were recruited from Outdoor Patient Department of Department of Pediatrics, AIIMS for this study. All infants were antiretroviral naïve and asymptomatic at the time of recruitment. After written informed consent from guardians, blood was drawn in 3-ml EDTA vials, plasma was aliquoted for plasma neutralization assays, viral RNA isolation, and viral loads. The study was approved by institute ethics committee of All India Institute of Medical Sciences.

Plasmids, viruses, monoclonal antibodies, and cells

Plasmids encoding HIV-1 env genes representing different clades, monoclonal antibodies and TZM-bl cells were procured from NIH AIDS Reagent Program. 10-1074 and BG18 were kindly provided by Dr. Michel Nussenzweig, Rockefeller University, USA. CAP256.09, CAP256.25 and b6 were procured from IAVI Neutralizing Antibody Centre, USA.

Neutralization assay

Neutralization assays were carried out using TZM-bl cells, a genetically engineered HeLa cell line that constitutively expresses CD4, CCR5 and CXCR4, and contains luciferase and β-galactosidase gene under HIV-1 tat promoter, as described before[8]. Neutralization studies included 47 heat-inactivated plasmas from infants, 19 bnAbs (PG9, PG16, PGT145, PGDM1400, CAP256.09, CAP256.25, 10-

1074, BG18, AIIMS-P01, PGT121, PGT128, PGT135, VRC01, N6, 3BNC117, PGT151, 35O22, 10E8) and 6 non-nAbs (b6, F105, 17b, 48d, A32, 447-52D). Briefly, envelope pseudoviruses were incubated in presence of serially diluted heat inactivated plasmas, bnAbs or non-nAbs for one hour. After incubation, freshly Trypsinized TZM-bl cells were added, with 25 µg/ml DEAE-Dextran. The plates were incubated for 48h at 37°C, cells were lysed in presence of Bright Glow reagent, and luminescence was measured. Using the luminescence of serially diluted bnAbs or plasma, a non-linear regression curve was generated and titres were calculated as the bnAb concentration, or reciprocal dilution of serum that showed 50% reduction in luminescence compared to untreated virus control. For plasma samples, percent neutralization for each plasma-virus combination was recorded as a breadth-potency matrix at a fixed dilution of 1/50: >80% neutralization received a score of 3, >50% a score of 2, >20% a score of 1, and <20 received a score of 0. Maximum cumulative score for a given plasma was 60, and neutralization score was given as the ratio of cumulative score for respective plasma to the maximum cumulative score, providing neutralization score on a continuous matrix of 0 to1, with values closer to 1 implying strong plasma neutralization activity. For epitope mapping, HIV-25710-2.43 pseudoviruses with key mutations within major bnAb epitopes were used[8], and greater than 3-fold reduction in ID50 titres were classifies as dependence. HIV-25710-2.43 mutants included N160K (V2-apex), N332K (V3-glycan), R456W (CD4 binding site), A512W-G514W (Interface) and W672L-F673L (MPER).

HIV-1 envelope sequences and phylogenetic analysis

HIV-1 envelope genes were PCR amplified from plasma viral RNA by single genome amplification and directly sequenced commercially. Individual sequence fragments of SGA amplified amplicons were assembled using Sequencher 5.4 (Gene Code Corporation). Nucleotide sequences were aligned with MUSCLE in MEGA X. Maximum-likelihood trees were computed with MEGA X using a general-time reversal substitution model incorporating a discrete gamma distribution with 5 invariant sites. Evolutionary divergence within each infant's SGA sequence was conducted in MEGA X and was calculated as number of base substitutions per site from averaging over all sequence pairs. Analyses were conducted using the Maximum Composite Likelihood model. The rate variation among sites was modelled with a gamma distribution (shape parameter = 5). This analysis involved 18 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous

positions were removed for each sequence pair (pairwise deletion option). There were a total of 2622 positions in the final dataset.

Nucleotide sequence accession numbers

The SGA amplified HIV-1 envelope sequences have been submitted to GenBank. Accession numbers will be provided once available.

Cloning of autologous HIV-1 envelope genes and production of replication incompetent pseudoviruses

Autologous replication incompetent envelope pseudoviruses were generated from AIIMS704, AIIMS706, AIIMS709 and AIIMS743 (elite neutralizers) as described previously[8]. Briefly, viral RNA was isolated from 140 µl of plasma using QIAamp Viral RNA Mini Kit, reverse transcribed, using gene specific primer OFM19 (5` - GCACTCAAGGCAAGCTTTATTGAGGCTTA – 3`) and Superscript III reverse transcriptase, into cDNA which was used in two-round nested PCR for amplification of envelope gene using High Fidelity Phusion DNA Polymerase (New England Biolabs). The envelope amplicons were purified, and ligated into pcDNA3.1D/V5-His-TOPO vector (Invitrogen). Pseudoviruses were prepared by co-transfecting 1.25 µg of HIV-1 envelope containing plasmid with 2.5 µg of an envelope deficient HIV-1 backbone (PSG3Δenv) vector at a molar ratio of 1:2 using PEI-MAX as transfection reagent in HEK293T cells seeded in a 6-well culture plates. Culture supernatants containing pseudoviruses were harvested 48 hours post-transfection, filtered through 0.4µ filter, aliquoted and stored at -80°C until further use. TCID50 was determined by infecting TZM-bl cells with serially diluted pseudoviruses in presence of DEAE-Dextran, and lysing the cells 48 hours post-infection. Infectivity titres were determined by measuring luminescence activity in presence of Bright Glow reagent (Promega).

Statistical analysis

Mann-Whitney U test and Kruskal-Wallis test were used for comparison of two and three parameters respectively. All statistical analyses were performed on GraphPad Prism 8. A p-value of <0.05 was considered significant.

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Author contributions

N.M designed the study, performed SGA amplification, pseudovirus cloning, and neutralization assays, analyzed data and wrote the manuscript. S.S and A.D contributed to SGA amplification, pseudovirus cloning, and neutralization assays. S.K expressed BG18, 10-1074 and AIIMS-P01 bnAbs, and edited the manuscript. R.S, B.K.D, R.L, and S.K.K, provided the samples from HIV-1 infected infants. R.L and S.K.K provided patient care and management. K.L designed the study, edited and finalized the manuscript.

Competing financial interests

The authors declare no competing financial interests.

References

1. Sok, D. & Burton, D. R. Recent progress in broadly neutralizing antibodies to HIV. *Nat. Immunol.* **19**, 1179–1188 (2018).
2. Kwong, P. D. & Mascola, J. R. HIV-1 Vaccines Based on Antibody Identification, B Cell Ontogeny, and Epitope Structure. *Immunity* **48**, 855–871 (2018).
3. Goo, L., Chohan, V., Nduati, R. & Overbaugh, J. Early development of broadly neutralizing antibodies in HIV-1-infected infants. *Nat. Med.* **20**, 655–658 (2014).
4. Simonich, C. A. *et al.* HIV-1 Neutralizing Antibodies with Limited Hypermutation from an Infant. *Cell* **166**, 77–87 (2016).
5. Kumar, S. *et al.* An HIV-1 broadly neutralizing antibody from a clade C infected pediatric elite neutralizer potently neutralizes the contemporaneous and autologous evolving viruses. *J. Virol.* (2018) doi:10.1128/JVI.01495-18.

6. deCamp, A. *et al.* Global panel of HIV-1 Env reference strains for standardized assessments of vaccine-elicited neutralizing antibodies. *J. Virol.* **88**, 2489–2507 (2014).
7. Kulkarni, S. S. *et al.* Highly complex neutralization determinants on a monophyletic lineage of newly transmitted subtype C HIV-1 Env clones from India. *Virology* **385**, 505–520 (2009).
8. Mishra, N. *et al.* Viral Characteristics Associated with Maintenance of Elite Neutralizing Activity in Chronically HIV-1 Clade C-Infected Monozygotic Pediatric Twins. *J. Virol.* **93**, (2019).
9. Makhdoomi, M. A. *et al.* Evolution of cross-neutralizing antibodies and mapping epitope specificity in plasma of chronic HIV-1-infected antiretroviral therapy-naïve children from India. *J. Gen. Virol.* **98**, 1879–1891 (2017).
10. Khan, L. *et al.* Identification of CD4-Binding Site Dependent Plasma Neutralizing Antibodies in an HIV-1 Infected Indian Individual. *PloS One* **10**, e0125575 (2015).
11. Ditse, Z. *et al.* HIV-1 Subtype C-Infected Children with Exceptional Neutralization Breadth Exhibit Polyclonal Responses Targeting Known Epitopes. *J. Virol.* **92**, (2018).
12. Doria-Rose, N. A. *et al.* New Member of the V1V2-Directed CAP256-VRC26 Lineage That Shows Increased Breadth and Exceptional Potency. *J. Virol.* **90**, 76–91 (2016).
13. Anthony, C. *et al.* Cooperation between Strain-Specific and Broadly Neutralizing Responses Limited Viral Escape and Prolonged the Exposure of the Broadly Neutralizing Epitope. *J. Virol.* **91**, (2017).
14. Moore, P. L. *et al.* Evolution of an HIV glycan-dependent broadly neutralizing antibody epitope through immune escape. *Nat. Med.* **18**, 1688–1692 (2012).
15. van den Kerkhof, T. L. G. M. *et al.* HIV-1 escapes from N332-directed antibody neutralization in an elite neutralizer by envelope glycoprotein elongation and introduction of unusual disulfide bonds. *Retrovirology* **13**, 48 (2016).
16. Keele, B. F. *et al.* Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 7552–7557 (2008).
17. Novitsky, V., Moyo, S., Wang, R., Gaseitsiwe, S. & Essex, M. Deciphering Multiplicity of HIV-1C Infection: Transmission of Closely Related Multiple Viral Lineages. *PloS One* **11**, e0166746 (2016).
18. Luan, H. *et al.* Dual Infection Contributes to Rapid Disease Progression in Men Who Have Sex With Men in China. *J. Acquir. Immune Defic. Syndr. 1999* **75**, 480–487 (2017).

19. Bhiman, J. N. *et al.* Viral variants that initiate and drive maturation of V1V2-directed HIV-1 broadly neutralizing antibodies. *Nat. Med.* **21**, 1332–1336 (2015).
20. Gao, Y., Tian, W., Han, X. & Gao, F. Immunological and virological characteristics of human immunodeficiency virus type 1 superinfection: implications in vaccine design. *Front. Med.* **11**, 480–489 (2017).
21. Kumar, A. *et al.* Infant transmitted/founder HIV-1 viruses from peripartum transmission are neutralization resistant to paired maternal plasma. *PLoS Pathog.* **14**, e1006944 (2018).
22. Bar, K. J. *et al.* Wide variation in the multiplicity of HIV-1 infection among injection drug users. *J. Virol.* **84**, 6241–6247 (2010).
23. Kishko, M. *et al.* Genotypic and functional properties of early infant HIV-1 envelopes. *Retrovirology* **8**, 67 (2011).
24. Voss, J. E. *et al.* Elicitation of Neutralizing Antibodies Targeting the V2 Apex of the HIV Envelope Trimer in a Wild-Type Animal Model. *Cell Rep.* **21**, 222–235 (2017).
25. Andrabi, R. *et al.* The Chimpanzee SIV Envelope Trimer: Structure and Deployment as an HIV Vaccine Template. *Cell Rep.* **27**, 2426-2441.e6 (2019).
26. Sheward, D. J. *et al.* HIV Superinfection Drives De Novo Antibody Responses and Not Neutralization Breadth. *Cell Host Microbe* **24**, 593-599.e3 (2018).
27. Wagner, G. A. *et al.* Intrasubtype B HIV-1 Superinfection Correlates with Delayed Neutralizing Antibody Response. *J. Virol.* **91**, (2017).
28. Basu, D. *et al.* HIV-1 subtype C superinfected individuals mount low autologous neutralizing antibody responses prior to intrasubtype superinfection. *Retrovirology* **9**, 76 (2012).
29. van Schooten, J. & van Gils, M. J. HIV-1 immunogens and strategies to drive antibody responses towards neutralization breadth. *Retrovirology* **15**, 74 (2018).
30. Havenar-Daughton, C., Lee, J. H. & Crotty, S. Tfh cells and HIV bnAbs, an immunodominance model of the HIV neutralizing antibody generation problem. *Immunol. Rev.* **275**, 49–61 (2017).
31. Moore, P. L. *et al.* Potent and broad neutralization of HIV-1 subtype C by plasma antibodies targeting a quaternary epitope including residues in the V2 loop. *J. Virol.* **85**, 3128–3141 (2011).
32. Landais, E. *et al.* HIV Envelope Glycoform Heterogeneity and Localized Diversity Govern the Initiation and Maturation of a V2 Apex Broadly Neutralizing Antibody Lineage. *Immunity* **47**, 990-1003.e9 (2017).

33. Landais, E. *et al.* Broadly Neutralizing Antibody Responses in a Large Longitudinal Sub-Saharan HIV Primary Infection Cohort. *PLoS Pathog.* **12**, e1005369 (2016).

34. Wibmer, C. K. *et al.* Viral escape from HIV-1 neutralizing antibodies drives increased plasma neutralization breadth through sequential recognition of multiple epitopes and immunotypes. *PLoS Pathog.* **9**, e1003738 (2013).

35. Bonsignori, M. *et al.* Analysis of a clonal lineage of HIV-1 envelope V2/V3 conformational epitope-specific broadly neutralizing antibodies and their inferred unmutated common ancestors. *J. Virol.* **85**, 9998–10009 (2011).

Figures and tables

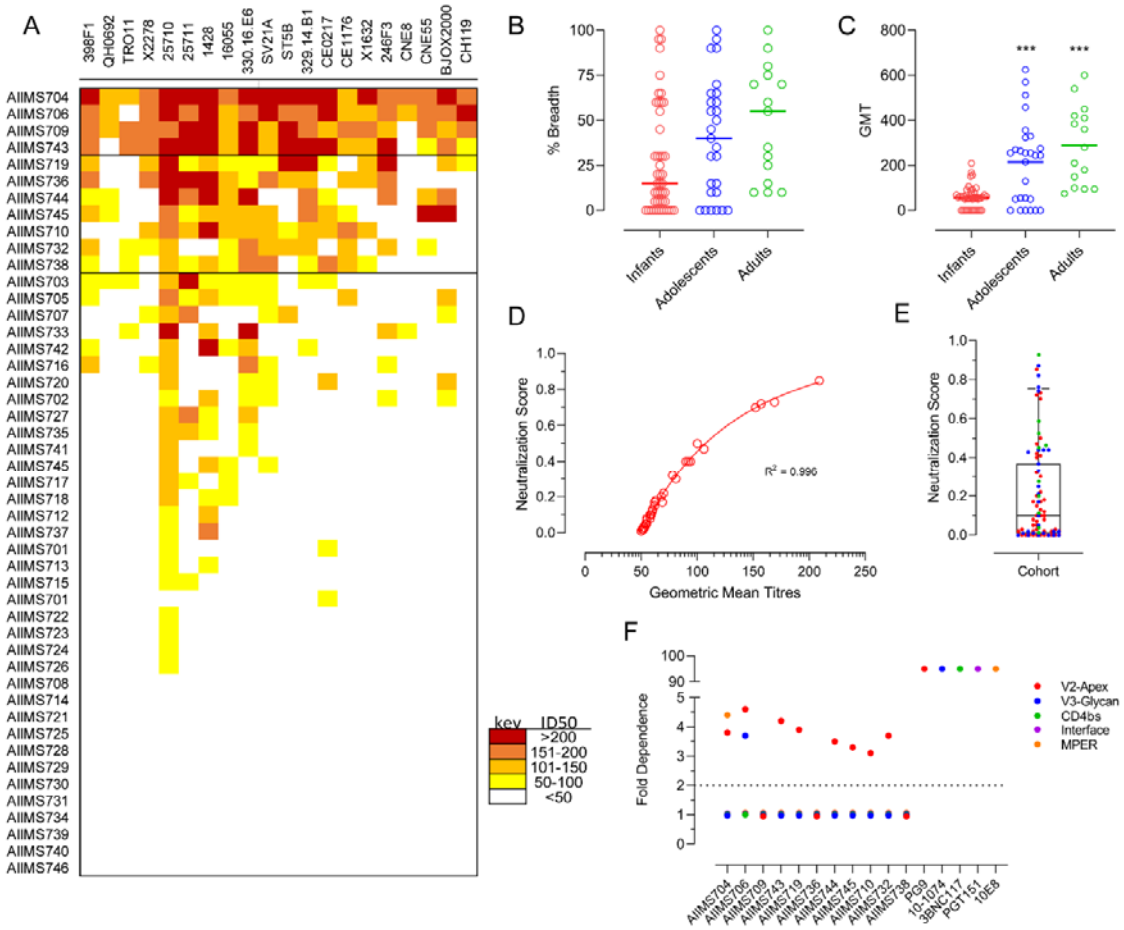


Figure 1 – Identification of plasma bnAb-inducing infants. (A) Heatmap representing HIV-1 specific neutralization titres (inverse plasma dilution) of plasma nAbs from 47 infant samples against the 20-virus global panel. (B and C) Comparison of breadth (pseudoviruses showing >50% neutralization at 1/50 plasma dilution) and geometric mean titres of infants with previously established cohorts of chronically infected children (labelled 'adolescents') and adults. (C) Modified neutralization scores detect geometric mean titres. (D and E) Validation of neutralization score to predict elite and broad neutralizers (red – infants, blue – adolescents and green – adults). (F) Epitope mapping done by mutant viruses for V2-apex (N160K), V3-glycan (N332A), CD4bs (R456W), Interface (A512W-G514W) and MPER (W672L-F673L). PG9 (V2-apex), 10-1074 (V3-glycan), 3BNC117 (CD4bs), PGT151 (Interface) and 10E8 (MPER) bnAbs were used as positive controls.

Category	Infant ID	Age (M)	Sex	CD4 Count	Plasma Viral Load (log)	% Breadth	GMT	Neutralization Score
Elite	AIIMS704	12	M	2592	5.59	100	209	0.85
	AIIMS706	9	M	1652	5.62	95	180	0.73
	AIIMS709	14	M	2142	5.33	95	167	0.72
	AIIMS743	28	M	1540	5.81	90	172	0.71
Broad	AIIMS719	19	F	1634	5.07	75	127	0.51
	AIIMS736	9	M	2595	5.89	65	161	0.49
	AIIMS744	14	M	1302	5.07	60	142	0.44
	AIIMS745	29	M	1642	4.59	65	123	0.43
	AIIMS710	24	F	1784	5.89	60	139	0.41
	AIIMS732	7	F	2105	6.04	60	105	0.37
	AIIMS738	11	M	1698	6.31	60	111	0.36

Table 1 – Demographics and neutralization profile of plasma bnAbs from infant elite and broad neutralizers.

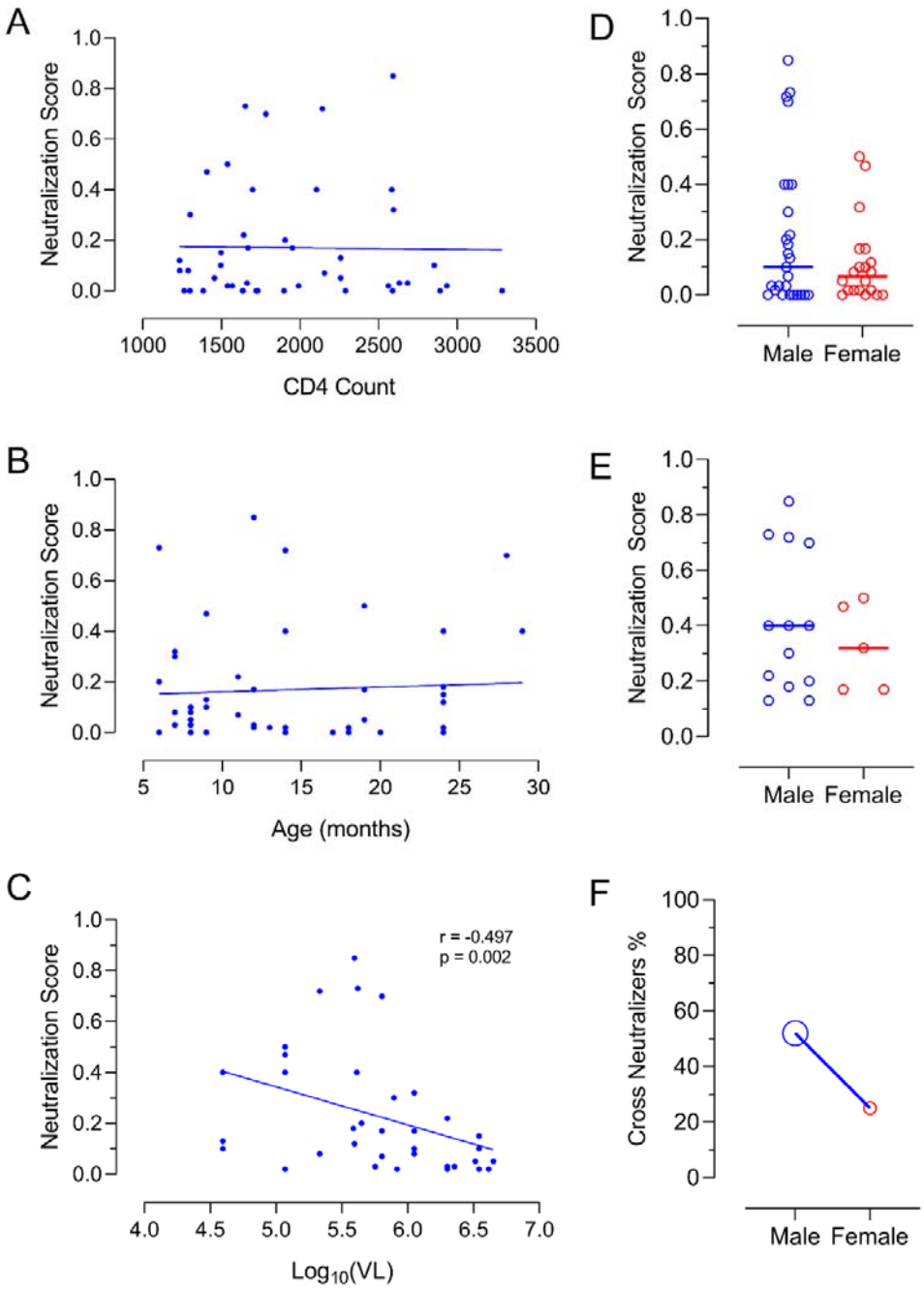


Figure 2 – Influence of viral load and gender on induction of plasma bnAbs. (A-C) Correlation between CD4 count, age and viral load with neutralization score. (D) Neutralization scores categorized for all 47 infants based on gender. (E) Neutralization score from cross-neutralizers (19/47) categorized based on gender. (F) Frequency of cross-neutralizers categorized based on gender in panel D.

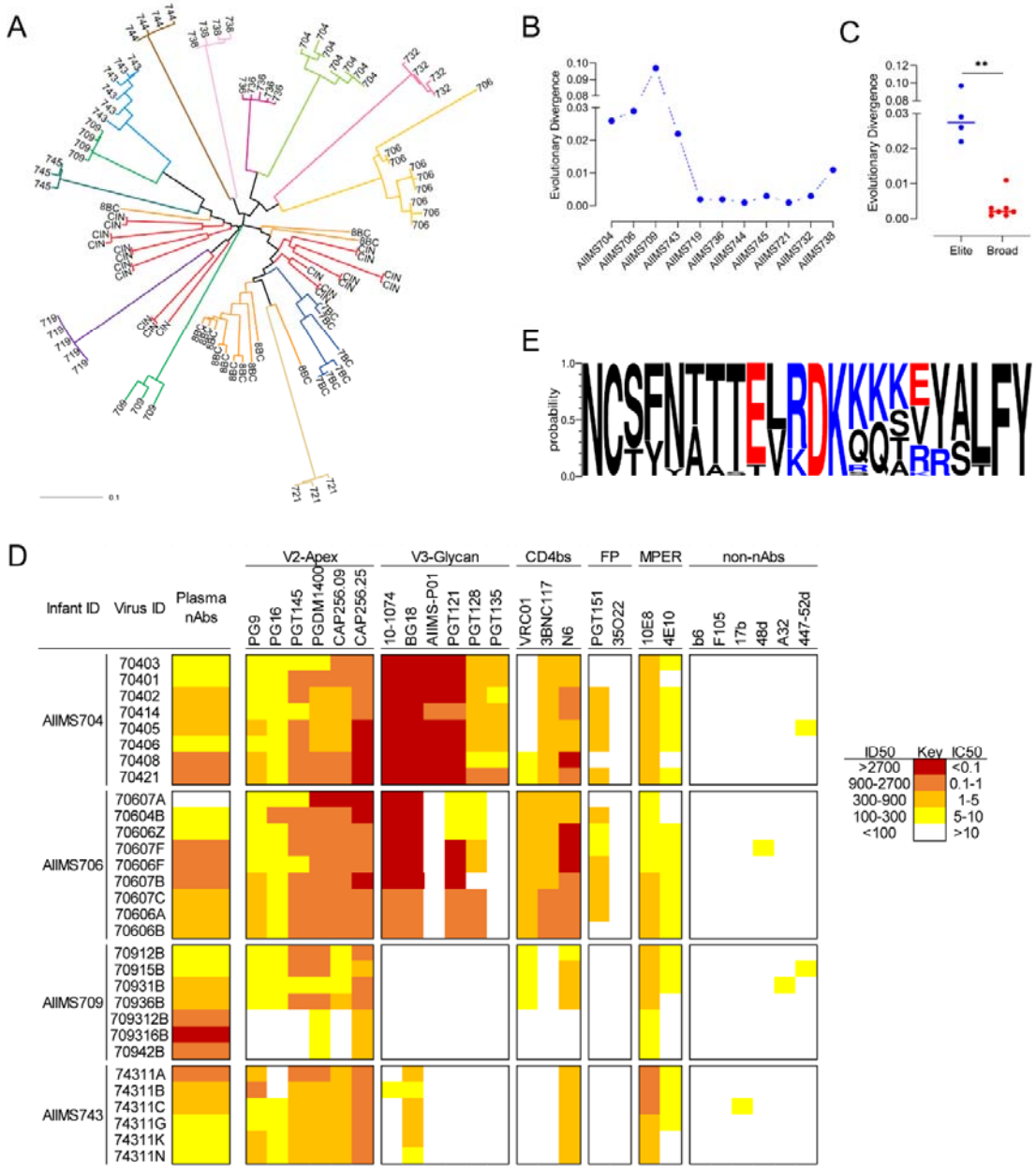


Figure 3 – Multivariant infection in infants with elite plasma neutralizing activity. (A) Phylogeny tree of SGA amplicons of circulating viral variants from infants with elite and broad plasma neutralizing activity. Two distinct branches for AIIMS704 (yellow green), AIIMS706 (yellow) and AIIMS709 (green) were observed. (B and C) Average evolutionary divergence among SGA amplicons for each infant. The number of base substitutions per site from averaging over all sequence pairs within each group are shown. (D) Susceptibility of functional pseudoviruses from elite neutralizers to their respective

autologous plasma antibodies (plasma nAbs), known bnAbs, and non-nAbs. (E) Amino acid frequency plots of V2-loop (HXB2 amino acids 156 – 177) from SGA amplicons of elite neutralizers.

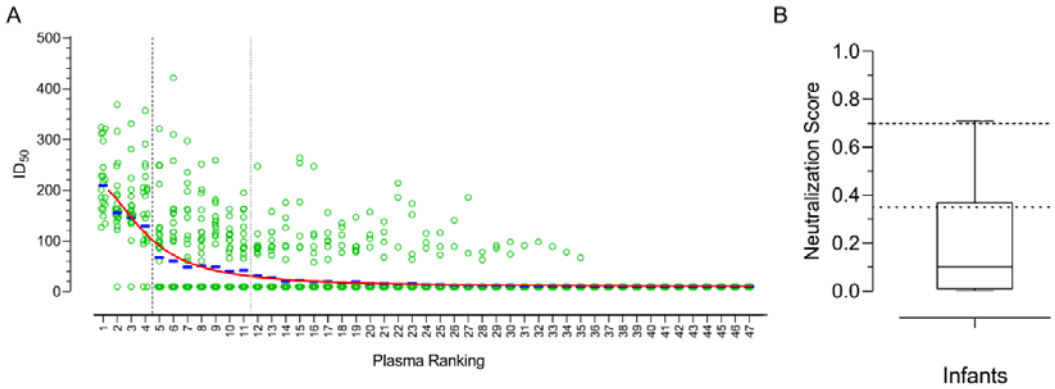
Virus name	Subtype	Tier	Country	Year	Accession
398F1	A1	2	Tanzania	NA	HM215312
246F3	A1C	3	Tanzania	NA	HM215279
QH0692	B	2	Trinidad and Tobago	1994	AY835439
TRO11	B	2	Italy	1995	AY835445
X2278	B	2	Spain	2007	FJ817366
1428	C	2	India	2000	EF117266
16055	C	2	India	1999	EF117268
25710	C	1B or 2	India	1999	EF117271
25711	C	1B or 2	India	1999	EF117272
330.16.E6	C	2	India	2016	MK076720
329.14.B1	C	2	India	2014	MK076593
SV21A	C	2	India	2017	NA
ST5B	C	2	India	2017	NA
CE0217	C	2	Malawi	2007	KC894109
CE1176	C	2	Malawi	2004	FJ444437
X1632	G	2	Spain	2004	FJ817370
CNE55	01_AE	3	China	2007	HM215418
CNE8	01_AE	2	China	2006	HM215427
BJOX2000	07_BC	2	China	2007	HM215364
CH119	07_BC	2	China	2004	EF117261

Supplementary Table 1 – 20-virus multiclade panel used for assessing the neutralization activity of plasma antibodies in HIV-1 infected infants of Indian origin. NA – not available.

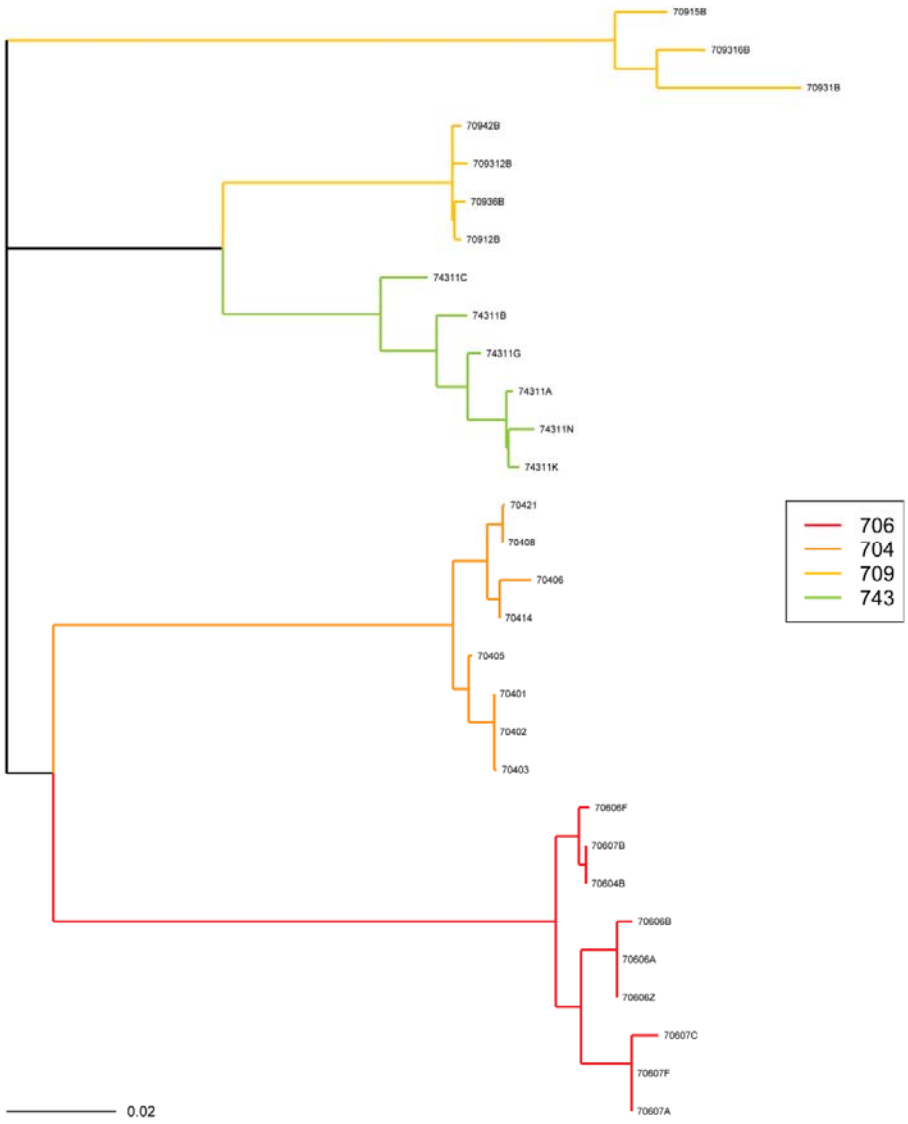
		Clade																								
		A	B			C										G	AC	AE		BC						
Infant ID	Age (M)	398F1	QH0692	TRO11	X2278	25710	25711	1428	16055	330.16.E6	SV21A	ST5B	329.14.B1	CE0217	CE1176	X1632	246F3	CNE8	CNE55	BJOX2000	CH119	MuLV	Cross-clade nAbs	% Breadth	Geometric Mean Titres	Neutralization Score
AIIMS704	12	245	134	127	164	324	321	216	162	221	314	297	201	312	149	228	176	172	186	228	186	<50	+	100	209	0.85
AIIMS706	9	174	129	<50	158	209	189	254	138	152	369	164	164	248	144	147	193	122	151	162	316	<50	+	95	180	0.73
AIIMS709	14	152	137	153	161	188	281	331	134	224	101	201	171	139	168	152	147	<50	159	148	153	<50	+	95	167	0.72
AIIMS743	28	196	<50	189	153	357	227	291	113	247	101	252	204	205	105	145	200	<50	99	152	94	<50	+	90	172	0.71
AIIMS719	19	112	<50	<50	127	321	86	124	61	89	97	249	249	87	<50	102	212	<50	<50	116	98	<50	+	75	127	0.50
AIIMS736	9	156	<50	<50	154	421	310	258	136	112	164	102	118	<50	107	118	161	<50	<50	<50	<50	<50	+	65	161	0.49
AIIMS744	14	68	61	<50	<50	297	160	235	103	214	<50	<50	150	94	<50	<50	198	<50	146	163	<50	<50	+	60	142	0.44
AIIMS745	29	101	76	<50	<50	194	89	103	117	144	118	<50	161	107	61	<50	<50	<50	201	251	<50	<50	+	65	123	0.43
AIIMS710	24	<50	<50	<50	148	181	110	259	141	137	132	94	112	117	176	126	<50	<50	<50	<50	<50	<50	+	60	139	0.41
AIIMS732	7	124	<50	78	88	112	<50	<50	84	163	102	149	114	<50	112	<50	102	<50	66	<50	<50	<50	+	60	105	0.37
AIIMS738	11	74	<50	85	<50	128	66	114	88	162	164	<50	87	195	147	99	<50	<50	<50	<50	<50	<50	+	60	111	0.36
AIIMS703	9	91	87	62	<50	114	247	88	74	84	78	<50	86	91	<50	<50	<50	<50	<50	<50	<50	<50	+	55	93	0.22
AIIMS705	6	89	<50	<50	<50	158	87	118	72	87	81	<50	<50	<50	102	<50	<50	<50	<50	118	<50	<50	+	45	99	0.21
AIIMS707	24	<50	<50	<50	64	126	176	<50	<50	<50	91	146	<50	<50	<50	<50	<50	<50	<50	88	<50	<50	+	30	109	0.18
AIIMS733	12	<50	<50	87	<50	254	<50	177	<50	264	<50	<50	<50	<50	<50	<50	110	89	<50	<50	<50	<50	+	30	147	0.17
AIIMS742	19	76	<50	<50	<50	114	<50	247	89	110	<50	<50	58	<50	<50	<50	<50	<50	<50	<50	<50	<50	+	30	103	0.17
AIIMS716	24	116	<50	<50	99	102	<50	<50	<50	156	64	<50	<50	<50	<50	<50	89	<50	<50	<50	<50	<50	+	30	101	0.15
AIIMS720	9	<50	<50	<50	<50	135	<50	<50	<50	77	91	<50	<50	112	<50	<50	<50	<50	<50	128	<50	<50	+	25	106	0.13
AIIMS702	24	<50	<50	<50	<50	89	<50	147	<50	88	82	<50	<50	<50	<50	<50	78	<50	<50	81	<50	<50	+	30	92	0.12
AIIMS727	8	<50	<50	<50	<50	112	157	88	<50	105	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	20	113	0.11
AIIMS735	8	<50	<50	<50	<50	118	138	68	<50	98	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	20	102	0.11
AIIMS741	9	<50	<50	<50	<50	186	<50	214	<50	88	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	15	152	0.11
AIIMS711	7	<50	<50	<50	<50	174	<50	152	64	<50	74	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	20	106	0.08
AIIMS717	8	<50	<50	<50	<50	115	97	<50	86	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	15	99	0.08
AIIMS718	11	<50	<50	<50	<50	116	<50	73	89	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	15	91	0.07
AIIMS712	19	<50	<50	<50	<50	89	<50	141	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	10	112	0.05
AIIMS737	8	<50	<50	<50	<50	76	<50	186	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	10	119	0.05
AIIMS747	7	<50	<50	<50	<50	78	<50	<50	<50	<50	<50	<50	<50	63	<50	<50	<50	<50	<50	<50	<50	<50	-	10	70	0.03
AIIMS713	12	<50	<50	<50	<50	91	<50	86	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	10	88	0.03
AIIMS715	8	<50	<50	<50	<50	98	74	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	10	85	0.03
AIIMS701	24	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	91	<50	<50	<50	<50	<50	<50	<50	<50	-	5	91	0.02
AIIMS722	13	<50	<50	<50	<50	98	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	5	98	0.02
AIIMS723	12	<50	<50	<50	<50	89	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	5	89	0.02
AIIMS724	14	<50	<50	<50	<50	78	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	5	78	0.02
AIIMS726	18	<50	<50	<50	<50	67	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	5	67	0.02
AIIMS708	18	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	0	0	0
AIIMS714	20	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	0	0	0
AIIMS721	6	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	0	0	0
AIIMS725	9	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	0	0	0
AIIMS728	8	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	0	0	0
AIIMS729	14	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	0	0	0
AIIMS730	18	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	0	0	0
AIIMS731	9	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	0	0	0
AIIMS734	24	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	0	0	0
AIIMS739	17	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	0	0	0
AIIMS740	24	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	0	0	0
AIIMS746	24	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	-	0	0	0

ID50	key
>200	
151-200	
101-150	
50-100	
<50	

Supplementary figure 1 – Plasma neutralization activity of infant plasmas against a 20-virus multiclade panel representing globally circulating HIV-1 viral diversity. ID50 values are color-coded based on the key provided with dark colors representing higher titres. Cross-clade neutralization implies the ability to neutralize a virus other than clade C (infecting clade in these infants). Geometric mean titres are calculated against the sensitive viruses. Neutralization scores were calculated based on a modified breadth-potency matrix. Murine Leukemia Virus (MuLV) was used as a negative control.



Supplementary figure 2 – Neutralization scores calculated based on modified breadth-potency matrix were predictive of geometric mean titres. (A) Infant plasmas are ranked based on geometric mean titres. Green dots represent individual ID50 values against the 20-virus panel, whereas the blue bar represents geometric mean titres, red line shows non-linear curve fit of modified neutralization score. Neutralization scores accurately predict geometric mean titres. (B) Quartile distribution of neutralization scores show 75th percentile at a cut-off of 0.35 and 90th percentile at 0.7. Elite neutralizers typically showed neutralization scores in the 90th percentile, whereas broad neutralizers had a neutralization score in the range of 75th to 90th percentile.



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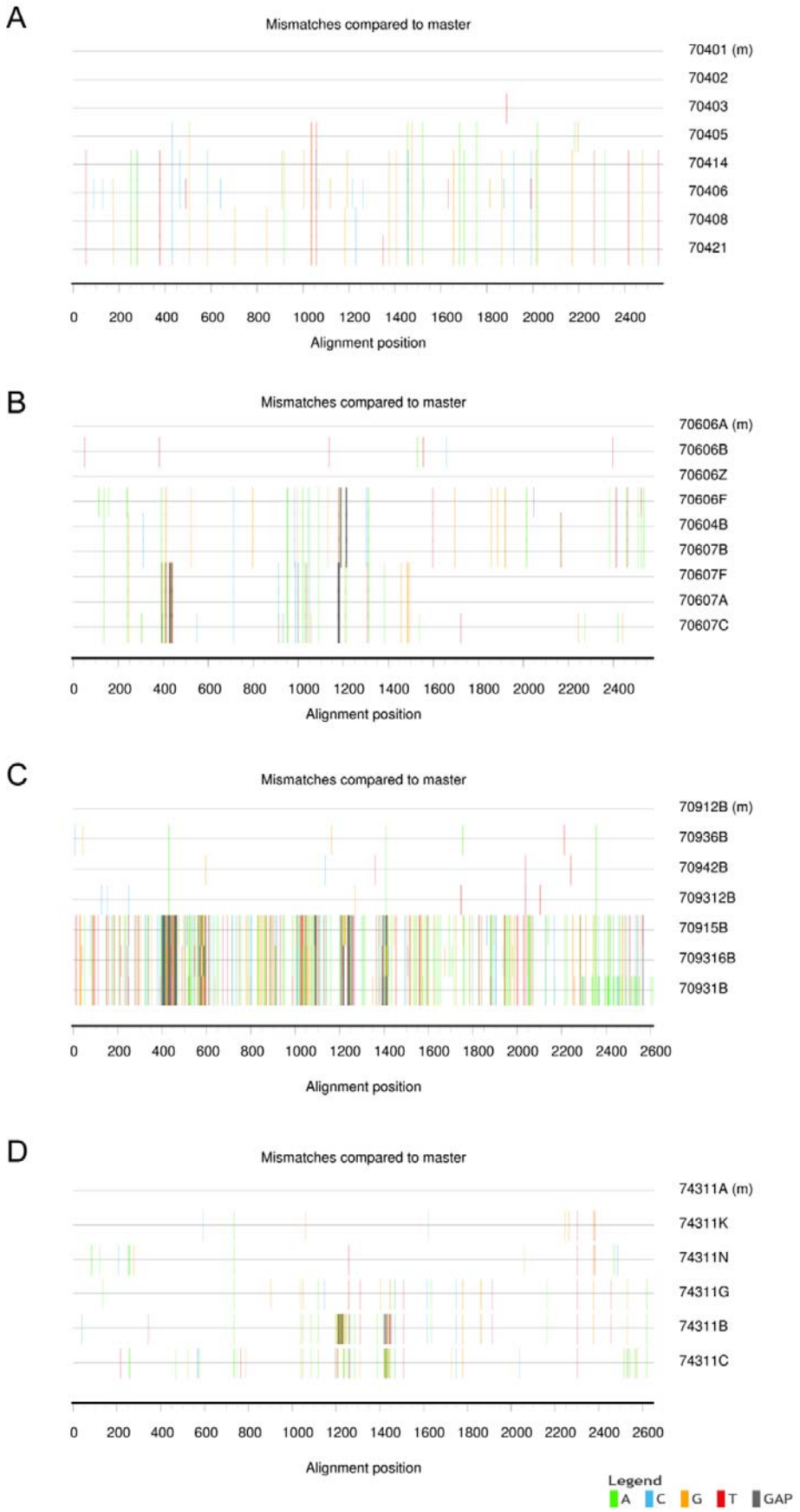
353 Supplementary figure 3 – Maximum-likelihood tree of SGA env sequences from infant elite
354 neutralizers. AIIMS704, AIIMS706, AIIMS709 SGA sequences shows distinct circulating viruses as
355 evident by distinct branches on the phylogeny tree suggesting multivariant infection. SGA sequences
356 from AIIMS743 showed branching more typical of a single variant infection evolving under immune
357 pressure.


A
Mismatches compared to master
70401 (m)
70402
70403
70405
70414
70406
70408
70421
0 200 400 600 800 1000 1200 1400 1600 1800 2000 2200 2400
Alignment position
B
Mismatches compared to master
70606A (m)
70606B
70606Z
70606F
70604B
70607B
70607F
70607A
70607C
0 200 400 600 800 1000 1200 1400 1600 1800 2000 2200 2400
Alignment position
C
Mismatches compared to master
70912B (m)
70936B
70942B
709312B
70915B
709316B
70931B
0 200 400 600 800 1000 1200 1400 1600 1800 2000 2200 2400 2600
Alignment position
D
Mismatches compared to master
74311A (m)
74311K
74311N
74311G
74311B
74311C
0 200 400 600 800 1000 1200 1400 1600 1800 2000 2200 2400 2600
Alignment position
Legend
A C G T GAP

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Supplementary figure 4 – Highlighter plots of gp160 env sequences from elite neutralizers. (A) AIIMS704, AIIMS706, and AIIMS709 highlighter plots suggest productive infection with three distinct viruses whereas AIIMS743 showed productive infection by one virus and the highlighter plot was typical of viruses evolving from one lineage.

Infant ID	Age (M)	Sex	CD4 Count	Plasma Viral Load (log)	Number of SGA Amplicon	env Gene Diversity	Unique Circulating Variants	Transmitted/Founder Viruses (Phylogenetic Estimate)
AIIMS704	12	M	2592	5.59	43	0.011	7	3
AIIMS706	9	M	1652	5.62	37	0.029	9	3
AIIMS709	14	M	2142	5.33	31	0.097	7	2
AIIMS743	28	M	1540	5.81	34	0.022	6	1
AIIMS719	19	F	1634	5.07	31	0.002	6	1
AIIMS736	9	M	2595	5.89	27	0.002	5	1
AIIMS744	14	M	1302	5.07	32	0.001	5	1
AIIMS745	29	M	1642	4.59	30	0.003	3	1
AIIMS721	24	F	1784	5.89	31	0.001	4	1
AIIMS732	7	F	2105	6.04	31	0.003	4	1
AIIMS738	11	M	1698	6.31	35	0.011	5	1

Supplementary Table 2 – Demographics and HIV-1 envelope analysis for multiplicity of infection in infant elite and broad neutralizers.