Non-induced Proviral Genome Characterization in Perinatal HIV Infection

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BACKGROUND

The Mississippi Child provides hope that HIV remission is possible for the >3 million perinatally-infected children in the world.

The latent HIV reservoir serves as a significant barrier to HIV remission, defined as undetectable viral load after cessation of antiretroviral therapy (ART).

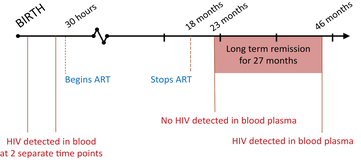
Traditionally, stimulation of cultured resting CD4+ T cells from HIV-infected individuals is used to induce HIV expression and measure the size of the latent HIV reservoir.

A recent study of chronically-infected adults identified replication-competent, non-induced provirus, however, suggesting the reservoir may be significantly larger than previously estimated (Ho et al 2013 Cell 155:540-51).

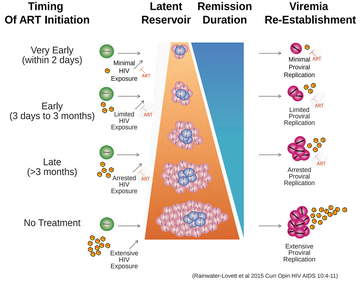
These non-induced proviral genomes (NIPG) serve as an infectious source of HIV, capable of rekindling viremia, and prevent cessation of ART in HIV-infected individuals.

Here, we characterize NIPG in perinatally-infected children and adolescents who initiated ART before 6 months of age and explore their role as a biomarker for HIV remission.

Mississippi Child: Timeline of Events



Begin ART earlier, restrict latent reservoir development



(Rainwater-Lovett et al 2015 Curr Opin HIV AIDS 10:4-11)

Early treated children typically rebound within 2 weeks of ART cessation

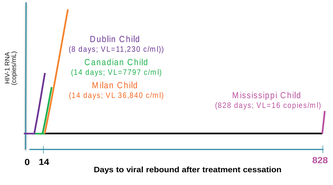


Table 1. Study Population

Subject	Age Virus Suppression (wks)	Current Age (yrs)	Duration of HIV Suppression (yrs)	# Cultured CD4+ T Cells ($x10^6$)	Replication-Competent HIV in Culture	Western Blot	# NIPG
0113.4	*NA*	10.6	*NA*	22	No	*NA*	20
0113.6	*NA*	10.8	*NA*	16	No	*NA*	12
0113.7	*NA*	11.1	*NA*	12	No	Ind	19
0102.1	6.7	13.5	13.4	30	No	Neg	52
0106.1	20.4	16.1	15.3	8.6	No	Ind	0
0107.1	16.4	11.4	11.1	22	**Yes**	Pos	22
0109.1	6.1	15.7	15.6	12	No	Neg	0
0110.1	7.1	15.3	15.1	15	No	Neg	2
0112.1	8.6	12.5	12.4	22	No	Ind	0
0113.1	8.4	11.1	11	6	No	Ind	1
0114.1	*NA*	8.2	*NA*	15	**Yes**	Pos	7
0115.1	7	5.9	5.8	4	No	Ind	0
0118.1	24.3	7	6.6	13	No	Ind	1
1042.3	11.4	17.7	17.5	15	No	*NA*	20
1042.4	11.4	18.4	18.2	27	No	Ind	3
1043.3	11.4	17.7	17.5	24	No	*NA*	4
1048.2	2.1	15.9	15.8	19	**Yes**	Neg	4
1115.1	18.9	20.5	20	24	No	Pos	23
1118.1	17.4	17.8	18	21	No	Pos	2
1368.1	9.5	4.3	4.1	9	No	Ind	6
9003.1	8.6	15	14.8	20	No	Neg	44
9004.1	12	6.4	6.15	40	No	Neg	0
9005.1	8.9	6.6	6.5	25	No	Ind	5
9008.1	11.1	15.2	15	23	**Yes**	Pos	11
Median	10.3	13	14.9	19.5			4.5

METHODS

Replication-competent HIV was induced through stimulation of resting memory CD4+ T cells in the quantitative co-culture assay and measured using the p24-antigen ELISA. Genomic and HIV DNA was extracted from each culture well using the QIAamp DNA Mini Kit (Qiagen). HIV DNA concentrations were measured in triplicate using primers against the *pol* gene by droplet digital PCR (BioRad). The ribonuclease P gene (RPP30) was used to adjust for the cellular equivalent of genomic DNA analyzed per reaction and report HIV DNA concentrations as *pol* copies per million cells.

Non-induced proviral genomes (NIPG) were detected in p24-negative culture wells by limiting dilution assay and single genome sequencing using previously described methods (Ho et al. 2013 Cell 155:540-51.)

Sequences were aligned in CodonCode Aligner v4.0.4. Hypermutation was determined using the Los Alamos National Laboratories Hypermut tool (http://www.hiv.lanl.gov), A neighbor-joining phylogenetic trees were produced in MEGA using theTamur-Nei 3-parameter model with 1000 bootstrap replicates and rooted using the HXB2 reference sequence in FigTree (http://tree.bio.ed.ac.uk/software/figtree).

HIV-specific antibodies were detected in plasma samples using the GS HIV-1/2 Plus O EIA kit (BioRad) per the manufacturer's instructions and the Cambridge Biotech HIV-1 Western blot kit (Maxim Biomedical).

The Mann-Whitney U-test was used to compare HIV DNA concentrations between p24-negative culture wells with and without NIPG and wells containing replication-competent HIV. Statistical analysis was conducted in R v3.1.0 (http://www.R-project.org).

NIPG Identification

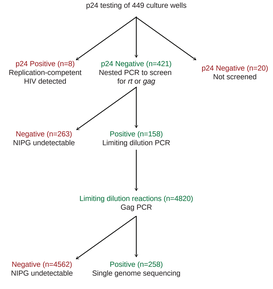


Figure 1. NIPG Distribution by Gag and Near-Full Length Sequences

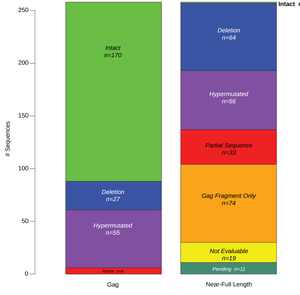


Figure 2. NIPG Distribution by Participant

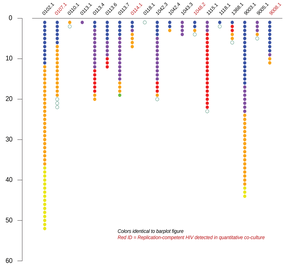


Figure 3. Location of Deletions in NIPG Sequences

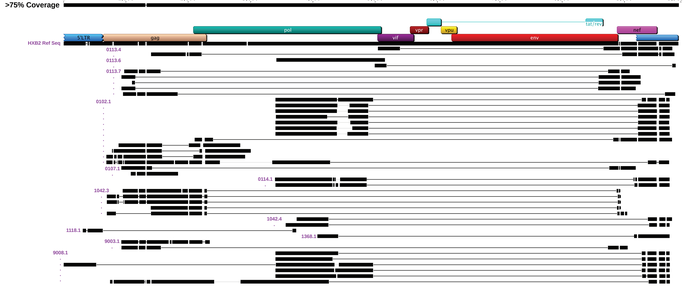


Figure 4a. Phylogenetic Tree of Gag Sequences

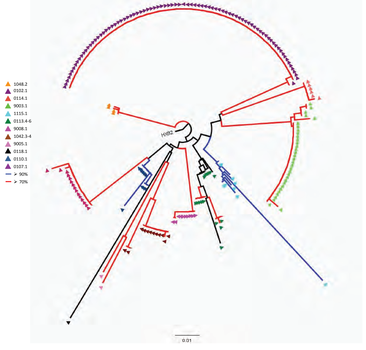


Figure 4b. Pairwise Distances between NIPG Gag Sequences

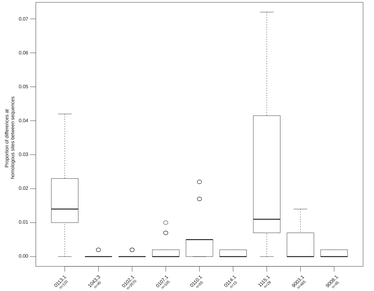


Figure 5. HIV-specific Antibody Detected by ELISA

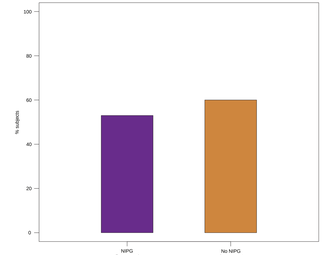


Figure 6. Western Blot Patterns from Plasma of Subjects with Replication-Competent HIV and NIPG from Culture Wells

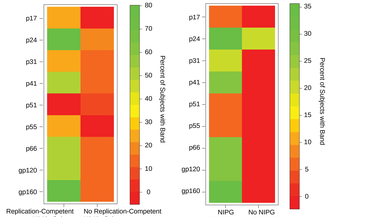


Figure 7. HIV DNA Concentrations by Presence of Replication-Competent HIV and NIPG from Culture Wells

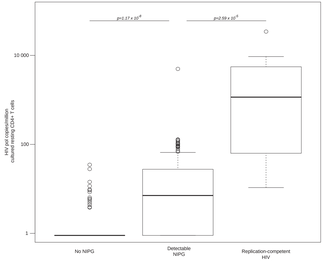
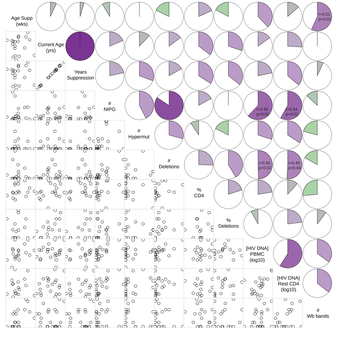


Figure 8. Correlations between Participant Characteristics and Viro-Immunologic Biomarkers



CONCLUSIONS

>99% of NIPG were replication-deficient in early-treated, perinatally-infected adolescents with long-term virologic suppression. This differs from chronically-infected adults in whom 12% of NIPG were replication-intact (Ho et al 2013 Cell 155:540-51).

Few replication-intact NIPG confirms the lack of detection of replication-competent HIV in the quantitative viral outgrowth assay and maintenance of low reservoir size with early treatment in perinatal infection.

Replication-deficient NIPG are clonal suggestive of proliferative expansion as a mechanism of maintenance of NIPGs (Wagner et al 2014 Science 345:570-3; Maldarelli et al 2014 Science 345:179-83; Cohn et al 2015 Cell 160:420-32) along with preferential clearance of cells with replication-intact provirus (Cohn et al 2015 Cell 160:420-32).

Reactivity to HIV proteins differed by detection of replication-competent HIV in the quantitative viral outgrowth assay and NIPG and correlated with age at virologic suppression.

The rarity of replication-intact NIPG suggests early ART initiation and/or long treatment duration is associated with profound depletion of replication-intact HIV-1 genomes.

These findings contribute to our understanding of HIV-1 persistence in perinatal infection with implications for strategies towards viral remission and cure for this population.

ACKNOWLEDGEMENTS

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