LACK OF COMPARTMENTALIZATION IN THE LATENT RESERVOIR OF BLOOD AND LYMPH NODES

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Background

- There are conflicting reports on the similarity of the HIV-1 latent viral reservoir (LVR) in the lymph node (LN) verses peripheral blood (PB) [1].
- Evaluate the HIV-1 LVR in the LN compared to PB and estimate the likelihood Compartmentalization between compartments.

Methods

- HIV+ individuals on ART with suppressed viral loads who were undergoing solid organ transplantation consented to have LN removed at the time of transplant, and PB and LN mononuclear cells (MC) were collected and isolated (n=10).
- CD4+ cells from matched PBMC and LNMC samples were plated in a novel quantitative viral induction assay (QVIA) that measures viral mRNA levels post-stimulation using qPCR in a limiting dilution set-up.
- Sequence data was obtained from positive wells (high mRNA production) using a validated site-directed next-generation sequencing of gp41, and prominent induced viral variants were identified (>2.5% of amplicons; Illumina Inc.).
- Subjects who had sequences obtained from >75% of their positive wells were analyzed[2] for compartmentalization.
- Neighbor-joining trees of all prominent patient sequences were inferred (Geneious prime), and identical variants were classified as 'replicates', it should be noted that due to the size of the sequences these may not be from clonal populations.
- A Bayesian model (IUPMBayes) was used to estimate the relative size of the LVR for each participant, as well as the proportion of the LVR made up by each variant for their respective compartments.
- Compartmentalization was assessed on samples using a Hudson based test for panmixia (non-parametric) and a branch length tree correlation coefficient (parametric)[3].

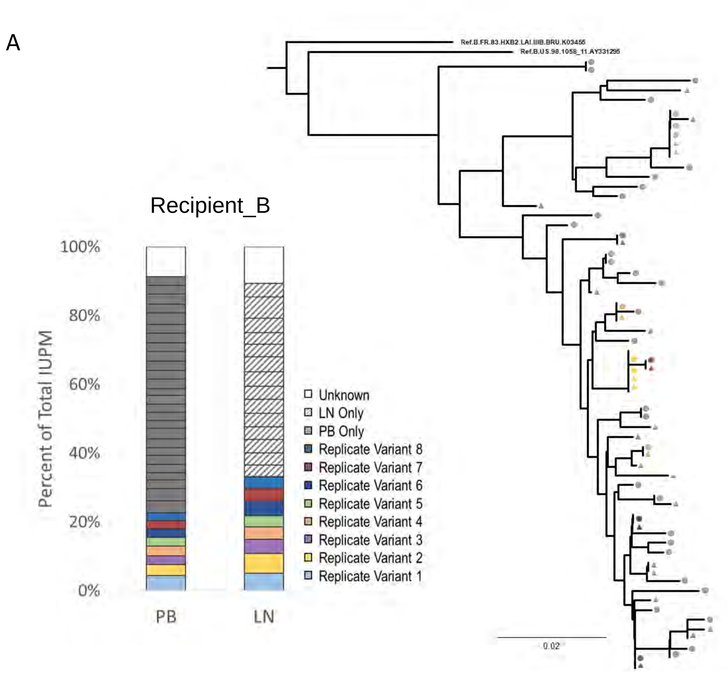
Results

- The estimated frequency of latently infected cells was a median of 10.56 in PB (IQR=4.59, 25.61) and 8.17 in LN (IQR=4.63, 21.67).
- Four individuals were found to have sufficient sequence coverage for analysis (median of 20.5 induced variants were identified in PB [IQR=5.5, 40.75] vs. 17.5 in LN [IQR=8.5, 22.75].)
- The estimated proportion of the LVR made up of variants that were replicated in the patient's matched compartment varied between compartments and between patients [median % LVR shared for PB=39.6% (IQR=78.0-25.8%) and LN=39.9% (IQR=63.85-30.6%).]

- Across all four HIV+ organ recipients on ART a median of 39.6% of the LVR found in the PB and 39.9% in the LN were found to have identical sequence replicates.
- No significant differences or divergence in PB and LN populations were found by statistical tests for compartmentalization.

Summary & Conclusions

- Characterizing the composition and any possible differences in anatomical compartments remains a crucial step to understanding the barriers to HIV cure.
- These data provide further evidence of intermingling and limited compartmentalization between the LN and PB, and support previous data that the LVR found in the blood can be a good representation of the LVR.



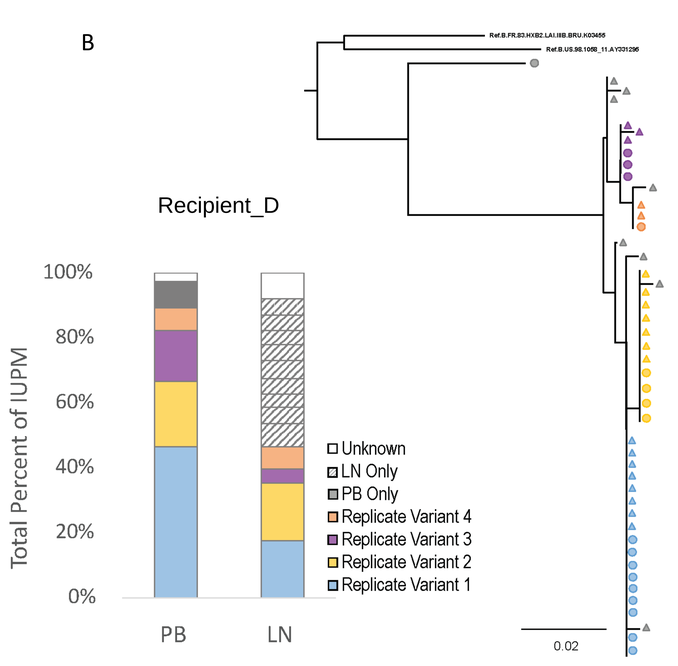


Figure: Neighbor-joining trees of distinct and replicate viral gp41 prominent species between PB(Circle) and LN(Triangle) compartments from induced viral RNA populations from QVIA positive wells of two patients. Bar graph coloring shows percent of the LVR made up by each variant. Replicate sequences shared between sites are shown with matched colors, solid grey sequences were detected only in PB and striped grey were found only in LN. A) The percentage shared variants for Recipient_B is PB= 22.6%, LN=33.2% B) The percentage shared variants for Recipient_D is PB= 89.4%, LN=46.5%.

Table 1. Summary of IUPMBayes in PB & LN

Patient ID	No. of Sequences obtained		Est. size of latent reservoir		No. of Induced Varients		No. of Shared Replicant Varients	Frequency of Reservoir Shared (%)	
	PB	LN	PB	LN	PB	LN		PB	LN
Recipient_A	30	99	30.14	25.99	43	23	17	43.75%	69.62%
Recipient_B	25	40	12.03	7.65	34	22	8	22.59%	33.20%
Recipient_C	10	10	3.09	3.63	7	7	2	35.46%	33.24%
Recipient_D	27	18	9.08	8.7	5	13	4	89.35%	46.53%

Table 2. Compartmentalization Results

Patient ID	Panmixia		Branch Length Correlation Coefficient	
	K*s	P-value	BLCC	P-value
Recipient_A	2.443	0.209	-0.004	0.488
Recipient_B	2.352	0.803	-0.044	0.975
Recipient_C	2.169	0.893	-0.061	0.908
Recipient_D	1.151	0.795	-0.009	0.645

References

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