Compartmentalized HIV-1 Is Found in the Semen of Men With and Without Urethritis

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Abstract #250

Background

- HIV-1 RNA can be found in the semen of most untreated, HIV-infected men[1].
- HIV-1 shed in the semen may represent local viral replication or migration into the genital tract from the periphery.
- Viral shedding is correlated with inflammatory processes, such as the presence of a concurrent STI infection. It is hypothesized that inflammation increases HIV-1 shedding in semen by recruiting CD4+ T cells into the genital tract[1].
- The male genital tract contains immune privileged sites that can support viral replication that is independent of the periphery, leading to the development of viral variants that are genetically distinct from virus in the blood (compartmentalization).
    - We have previously reported the identification of macrophage-tropic HIV-1 in a compartmentalized, semen-derived lineage[2].
- **In the current study, we examine how the presence of a concurrent STI infection affects the development of compartmentalized HIV-1 lineages in the genital tract**.
    - We hypothesize that inflammation due to STI-associated urethritis increases migration of HIV-infected cells into the genital tract, causing HIV-1 in the semen to be genetically similar to virus in the blood (equilibrated).
    - In the absence of inflammation, HIV-1 replication can occur independently in the genital tract creating lineages that are genetically distinct from that in the periphery (compartmentalized)

Materials and Methods

Source of Samples: Paired blood and semen samples were collected from HIV-1 infected, antiretroviral therapy naïve men in Lilongwe, Malawi with and without symptomatic urethritis.

Deep Sequencing of HIV-1 Env (V1-V3): HIV-1 RNA was extracted from the samples and cDNA was synthesized. Each cDNA was tagged with a unique 11 base pair Primer ID. This Primer ID 'tag' is maintained throughout subsequent amplification steps and reduces PCR-mediated recombination errors. Approximately 500 bases of the HIV-1 env V1-V3 region were deep sequenced using the MiSeq platform. Phylogenetic trees were then constructed using sequences from the blood and semen and examined for the presence of compartmentalization in the genital tract.

Results

Blood and Semen-Derived HIV-1 *Env* Sequences Can Be Equilibrated in Men With Urethritis

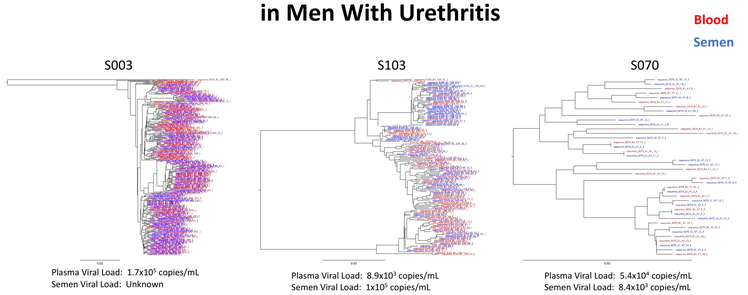


Figure 1. Blood and semen-derived HIV-1 V1-V3 sequences can be equilibrated in men with urethritis. Deep sequencing of paired blood and semen sequences from men experiencing symptomatic urethritis reveals genetically similar HIV-1 envelope (V1-V3) sequences in both the blood and semen. Blood-derived sequences are shown in red, semen-derived sequences are shown in blue.

Compartmentalized HIV-1 *Env* Sequences Can Be Found in the Semen of Men With Urethritis

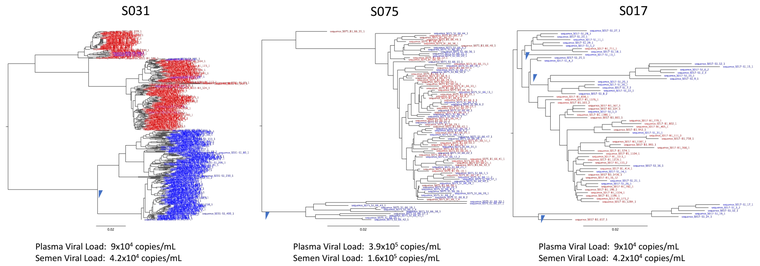


Figure 2. Compartmentalized HIV-1 *Env* sequences are found in the semen of men with urethritis. Midpoint-rooted phylogenetic trees for three representative individuals are shown. Compartmentalized nodes are circled. Blood-derived sequences are shown in red, semen-derived sequences are shown in blue.

Blood and Semen-Derived HIV-1 *Env* Sequences Can Be Equilibrated in Men Without Urethritis

Figure 3. Blood and semen-derived HIV-1 *Env* (V1-V3) sequences can be equilibrated in men without urethritis. Deep sequencing of men without symptomatic urethritis (no STI) reveals equilibrated viral populations in a subset of individuals. Blood-derived sequences are in red, semen-derived sequences are in blue. An equal number of blood and semen-derived sequences was used for each phylogenetic tree.

Compartmentalized HIV-1 *Env* Sequences Can Be Found in the Semen of Men Without Urethritis

Figure 4. Compartmentazlied HIV-1 *Env* (V1-V3) sequences are found in the semen of men without urethritis. A subset of men without symptomatic urethritis (no STI) display varying degrees of viral compartmentalization in the semen. Blood-derived sequences are shown in red, semen-derived sequences are shown in blue.

Similar Viral Loads and CD4 Counts Are Observed in Individuals Who Display Compartmentalized or Equilibrated Viral Lineages

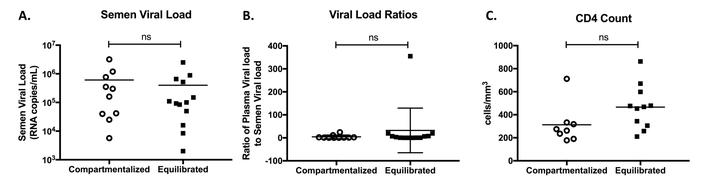


Figure 5. No difference in semen viral load or CD4 count in compartmentalized or equilibrated individuals. Patients were divided into groups based on whether the HIV-1 sequences in their blood and semen were compartmentalized or equilibrated. Unpaired t tests were used to examine the difference in semen viral load, viral load ratio, and CD4 count between the groups. No statistically significant difference was observed.

Compartmentalized Viral Lineages Are Found in 41% of Men With or Without Urethritis

Group	Average Plasma Viral Load	Average Semen Viral Load	CD4 Count	No. of *env* sequences	Compartmentalization
STI (n=16)	$3.24x10^5$	$2.53x10^{5\,a}$	393[c]	83	7/16 (44%)
No STI (n=8)	$1.5x10^6$	$9.35x10^5$	415[d]	42	3/8 (38%)

[a]average of 15 participants, [c]average of 12 participants, [d]average of 7 participants

Table 1. Summary of the samples analyzed. Out of 24 samples analyzed, we observed varying degrees of compartmentalization in 41% of men, regardless of whether or not they had urethritis. Thus, it appears factors other than inflammation influence the development of compartmentalized viral lineages.

Conclusions

- We observed varying degrees of compartmentalization in the genital tract of approximately 40% of men analyzed, regardless of the presence or absence of urethritis.
- No differences were observed in semen viral load or CD4 count in men displaying compartmentalized viral lineages.

References

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