Background

- 52% of people living with HIV-1 are females, yet they only represent ~11% of participants in HIV-1 cure studies.
- The latent viral reservoir, composed primarily of HIV-1 infected resting CD4+ T cells (cCD4), is the barrier to achieving HIV-1 cure.
- Females differ in immune responses to HIV-1 infection, including elevated in vivo expression of IFN-stimulated genes, elevated T cell responses, and decreased markers of immune exhaustion.
- Current cure strategies are focused on (a) permanently silencing the latent viral reservoir, or (b) eradicating the reservoir through the combined use of latency reversal agents and PD-1 antagonists.

Methods

- HIV-1 infected adults who were virally suppressed (<40 copies/mL) for over 1 year at time of enrollment (n = 90; female = 57, male = 33) were recruited from Rakai Health Sciences Program in Uganda.
- PBMCs were isolated from participant blood samples (180 mL) and used for the Quantitative viral outgrowth assay (cCD4 activation with PHA and γ-irradiated allogenic PBMC + limiting dilution culture) to identify latent HIV-1 reservoir outgrowth (IUPM) on clinical, and immunological covariates in females and males.
- Soluble immune markers were quantified using an 8-plex custom Human Ultra-Sensitive kit (Meso Scale Discovery).
- Univariate regression analysis and stepwise regression using R’s MASS package “stepAIC” function.

Results

Table 1. Clinical and immunological characteristics of HIV-1 positive Ugandans females and males.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Females (n = 57)</th>
<th>Males (n = 33)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41.1 (37.4, 47.2)</td>
<td>44.2 (40.3, 47.1)</td>
<td>0.15</td>
</tr>
<tr>
<td>CD4 T cell count</td>
<td>41.0 (13.0, 51.1)</td>
<td>11.5 (21.2, 68.9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CD4 T cell count (IQR)</td>
<td>3.88 (2.58, 4.19)</td>
<td>3.06 (2.51, 3.79)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Figure 2. Differences in (A) frequency of cCD4 that produce replication competent virus when stimulated ex vivo, by the QVOA, and (B) frequency of cCD4 that contain HIV-1 gag DNA between females (n=57) and males (n=33). Adjusted analysis controls for age, pre-ART viral load, nadir CD4 T cell count, duration of ART, instances of transiently detectable viremia (proportion of on-ART viral loads >400 copies/ml, and CD4 T cell count at the time of QVOA.

Conclusions

- Females have reduced viral outgrowth, but similar levels of total HIV DNA compared to males.
- This could be due to (1) a higher proportion of defective proviruses, and/or (2) a lower probability of provirus reactivation after ex vivo T cell stimulation in females compared to males.
- Correlates of reservoir size differ between males and females:
  - In males, the most significant correlate of reservoir size is expression of PD-1 on CD4 T cells, as previous reported in predominantly male North American cohorts (i.e. Chomont Nat Med 2009).
  - In females, reservoir size correlated positively with pre-ART viral load and negatively with time on ART, but not PD-1 expression.

Many thanks to the women and men from the Rakai Health Sciences Program for their dedicated participation in HIV research.