Long Terminal Repeat (LTR) region contains numerous transcription factors (TF) binding sites and its transcription by cellular and viral proteins is essential for provirus production. It has been shown that genetic variation within HIV-1 LTR on specific TF binding sites is associated with an increase in the size of the reservoir (Alter et al., 2010). Genetic diversity of HIV-1 LTR has only been assessed in previous studies within HIV group A (Akyi et al., 1994; Hamilton et al., 2004), one of the two epidemiological groups of HIV with the group B.

OBJECTIVE

The aim of this study was to assess the genetic diversity among HIV-2 LTR and its association with the size of the viral reservoir among HIV-2 antiretroviral naïve patients.

METHODS

• We included all antiretroviral-naïve patients from the French National Agency of Recherche sur le Sida et les hépatites virales (ANRS) HIV-2 CO5 cohort. For each patient, whole blood samples were collected. Peripheral Blood Mononuclear Cells (PBMC) were isolated from whole blood by Ficoll® (Eurobio) and DNA was extracted using QIA紡DNA Mini Kit (Clagen). HIV-2 total DNA quantification was performed by an in-house technique (Bertine et al., 2017), with a limit of quantification (LOQ) of 5 copies/PCR. Plasma viral load was measured on 140 strains with a limit of quantification of 20 copies/ml. LTR binding sites were analyzed by PhoMap® for group determination based on phylogenetic tree inferred with the maximum likelihood method with a General Time Reversible model (with gamma = 4) and verified by 500 bootstraps. Intragenic genetic distances were determined using PHD with a combination with AIDS and/or EHO reference strains. Bio-informatic research of putative TF binding sites was performed with MatInspector®. LTR binding transcriptional activity was assessed on HEK293T cells transfected by 50 ng of plasmid encoding the lucerase gene under the expression of different LTRs (pLTR-Luc). Luciferase activity was measured 24 hours after transfection, with the Bright-Glo (Lucerase Assay®, Promega). Statistical analysis was performed in R using Mann-Whitney and Fisher tests.

RESULTS

• LTR 2 region was successfully sequenced in 65 patients’ samples: 27 group A and 38 group B.

Among them, 8 had a plasma viral load ≥40 copies/ml. Demographic and biological characteristics of patients are summarized in Table 1.

Table 1: Patients’ characteristics.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (median)</th>
<th>Gender</th>
<th>Race</th>
<th>EHO status</th>
<th>LOQ results</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>37.9 (IQR=27)</td>
<td>Female</td>
<td>Black</td>
<td>Present</td>
<td>27.3 (IQR=12)</td>
</tr>
<tr>
<td>B</td>
<td>37.9 (IQR=31)</td>
<td>Male</td>
<td>White</td>
<td>Absent</td>
<td>33.2 (IQR=28)</td>
</tr>
</tbody>
</table>

Results on 65 patients samples: 27 group A and 38 group B

• Mean genetic variation within the LTR was significantly higher in group B than in group A (35.0% versus 13.2%, p < 0.002).

For both groups, the mean genetic distance was significantly higher in US region than in F-US region (58.6% versus 5.3%, p < 0.002) (Figure 2).

• Group B strains exhibited an increased genetic variability in the region corresponding to nucleotides 374 to 508 in the reference strain (ΔR) which includes most of known LTR binding sites (ΔR=10% versus 5.3% for group A, p=0.001).

• Next generation sequencing analysis of all 65 patients indicated that 50% of patients had at least one nucleotide substitution within the LTR region.

• The highest genetic variability and also the difference in the subregion Flur patients had a significantly lower frequency of HIV-2 DNA quantifiable compared to group B patients.

• In vitro transcriptional activity of LTR strain was higher in group B than in group A (p<0.001).

• The binding activity of LTR strain was higher in group B than in group A (p<0.001).

• Differences between HIV-2 group A and B need to be studied further, especially to determine if these differences within LTR are associated with the size of the viral reservoir in HVMC.

REFERENCES


