Characterization of Variable and constant regions of the Env protein of HIV-1 subtype C obtained from patients at different disease stages in Sub-Saharan Africa: mechanisms for viral escape?

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6th HIV Transmission Workshop
July, the 14th, 2011
Background 1: Characterization of Env variable sequences

- HIV-1 C is the prevalent subtype in sub-Saharan Africa and the most spread HIV-1 virus in the world.

- Several studies most carried out on subtype B, highlighted the importance of HIV-1 gp120 variable regions in virus infectivity and immune escape. In particular, characteristics such as sequence amino acid length and presence of putative N-glycosilation sites (PNGS) seem to be correlated with gp120 biological and antigenic properties.

- HIV variants isolated from recently infected patients can be more sensitive to antibody-mediated neutralization and display shorter V1-V2 loops than variants isolated during chronic infection, that, instead, display longer V1-V2 sequences and are more resistant to antibody-mediated neutralisation.

- In addition, a lower PNGS number has been found in gp120 from virus isolates obtained from recently infected subjects.
Background 2: Characterization of Env constant sequences

• Few Studies on HIV-1 clade C revealed resistance to neutralization by MAb 2G12, recognizing glycans, and identified a NAbs major target in the C3-V4 region.

• The HIV-1 glycoprotein Env binds to the target cells through cellular CD4 and chemokine receptors. V3 loop interacts with the chemokine receptors, whereas some selected residues in C3 and in C4 Env are critical for CD4 binding.

• The C3 domain of HIV-1 subtype C could play a role in neutralization escape, in particular through the α2-helix, where most of the sites under positive selection are located.

• A net electric charge change could drive the neutralization escape of HIV-1 virus.
Aim of the study

Characterization of gp120 variable and the C3 and C4 constant regions from HIV-1-clade-C variants isolated from patients at different disease stage for:

- Sequence lengths
- Number of PNGS
- Electric charge
- Presence of sites under positive selective pressure
### Classification of patients in disease stages

<table>
<thead>
<tr>
<th></th>
<th>Number of samples</th>
<th>A.I.</th>
<th>CD4 range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recent Infection</strong> (RI)</td>
<td>24</td>
<td>≤0.80</td>
<td>Not applicable</td>
</tr>
<tr>
<td><strong>Chronic Infection</strong> (CI)</td>
<td>24</td>
<td>&gt;0.80</td>
<td>418 - 767</td>
</tr>
<tr>
<td><strong>Late Infection</strong> (LI)</td>
<td>24</td>
<td>&gt;0.80</td>
<td>7 -199</td>
</tr>
</tbody>
</table>

Al ≤ 0,80 identifies a recent infection
Genomic subtyping of variants isolated from patients

V3-V5-based phylogenetic tree

Presented at the 6th Int. Workshop on HIV Transmission, 14 – 15 July 2011, Rome, Italy
Amino acid sequence lengths of V1, V2, V4 and V5 from patients at different infection stages

V1

V2

V4

V5

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Number of PNGSs in V1, V2, V4 and V5 regions from patients at different infection stages

V1

p-value 0.0121

V2

V4

V5

Number of PNGSs in V1, V2, V4 and V5 regions from patients at different infection stages

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Analysis of PNGSs variations in V1V2, V4 and V5 regions

Presented at the 6th Int. Workshop on HIV Transmission, 14 – 15 July 2011, Rome, Italy
Results variable regions

- **V1 and V4 domains** show similar behaviour with significant aminoacidic sequence length increases from recent to chronic infection. Conversely, in the passage from chronic to late infection, a reduction of amino acid number is found in both V1 and V4 regions.

- **V2** showed an opposite behaviour for aminoacidic sequence lengths without any significance, while **V5 remains quite constant in all stages of infection.**

- **V1, V2 and V4 domains** show an increase of PNGS number from recent to chronic infection. Only in **V1** there is a statistically significant increase of number of PNGS in recent versus chronic infection ($p=0.0121$).

- Instead, **V5 remains constant in PNGS number in all stages of infection.**

- **Shifting of PNGS occurs only in some restricted “hot-spots” regions,** almost all in the C-terminal of variable regions with the exception of the V5.
PNGS variations in V3-C3 and C4 regions

Recent Infection

Chronic Infection

Late Infection

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Amino acids under positive selection in V3, C3 and C4 regions

a) dN/dS Ratio >1 in V3 for single codon

<table>
<thead>
<tr>
<th>Aminoacid</th>
<th>Hxb2 ref number</th>
<th>V3 JR-FL aminoacid</th>
<th>JR-FL number</th>
<th>RI</th>
<th>CI</th>
<th>LI</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>300</td>
<td>5</td>
<td>300</td>
<td>3.056+0.699</td>
<td>3.056+0.690</td>
<td>4.676+1.223</td>
</tr>
<tr>
<td>14</td>
<td>309</td>
<td>12</td>
<td>307</td>
<td>-</td>
<td>3.019+0.718</td>
<td>4.676+1.228</td>
</tr>
<tr>
<td>27</td>
<td>322</td>
<td>25</td>
<td>320</td>
<td>3.050+0.707</td>
<td>3.050+0.696</td>
<td>-</td>
</tr>
</tbody>
</table>

b) dN/dS Ratio >1 in C3 for single codon

<table>
<thead>
<tr>
<th>Aminoacid</th>
<th>Hxb2 ref number</th>
<th>RI</th>
<th>CI</th>
<th>LI</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>335</td>
<td>4.711+0.995</td>
<td>3.507+0.122</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>336</td>
<td>4.902+0.598</td>
<td>3.507+0.118</td>
<td>7.808+1.550</td>
</tr>
<tr>
<td>6</td>
<td>337</td>
<td>4.864+0.703</td>
<td>3.505+0.138</td>
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</tr>
<tr>
<td>12</td>
<td>343</td>
<td>4.902+0.599</td>
<td>3.507+0.120</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>344</td>
<td>4.901+0.600</td>
<td>3.507+0.118</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>346</td>
<td>4.902+0.599</td>
<td>3.507+0.118</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>347</td>
<td>-</td>
<td>3.505+0.143</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>350</td>
<td>4.885+0.648</td>
<td>3.507+0.118</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>352</td>
<td>-</td>
<td>3.494+0.220</td>
<td>-</td>
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<tr>
<td>29</td>
<td>360</td>
<td>4.902+0.598</td>
<td>3.504+0.145</td>
<td>-</td>
</tr>
<tr>
<td>31</td>
<td>362</td>
<td>4.902+0.598</td>
<td>3.507+0.118</td>
<td>7.807+1.552</td>
</tr>
<tr>
<td>33</td>
<td>364</td>
<td>4.902+0.598</td>
<td>3.507+0.120</td>
<td>7.808+1.550</td>
</tr>
</tbody>
</table>

c) dN/dS Ratio >1 in C4 for single codon

<table>
<thead>
<tr>
<th>C4 aminoacid</th>
<th>Hxb2 number</th>
<th>RI</th>
<th>CI</th>
<th>LI</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>429</td>
<td>2.935+0.911</td>
<td>5.321+1.659</td>
<td>2.905+0.591</td>
</tr>
<tr>
<td>28</td>
<td>446</td>
<td>-</td>
<td>-</td>
<td>2.893+0.604</td>
</tr>
</tbody>
</table>
Aminoacids under positive selective pressure in C3 and C4 in gp120 bound to CD4

Recent

Chronic

Late

C3
C4

C3
C4

C3
C4

C3
C4

C3
C4

C3
C4

C3
C4

C3
C4

gp120 in grey, CD4 in green, PS sites in violet, and aminoacids critically for CCR5 in red

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Aminoacids under positive selective pressure in V3 in gp120 bound to CD4 and CCR5

gp120 in grey, CD4 in green, PS sites in violet, and aminoacids critically for CCR5 in red

d) Zoomed view in chronic infection

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Correlation of positive electric charges between C3 and V4 and between C3 and V5

**C3-V4**

- **RI**
  - Corr. Coeff. = 0.0825
  - p = not significant

- **CI**
  - Corr. Coeff. = -0.59199
  - p = 0.0023

- **LI**
  - Corr. Coeff. = -0.0941
  - p = not significant

**C3-V5**

- **RI**
  - Corr. Coeff. = 0.4974
  - p = 0.0134

- **CI**
  - Corr. Coeff. = 0.2388
  - p = not significant

- **LI**
  - Corr. Coeff. = 0.4863
  - p = 0.016

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Contact map of C3-V4 and C3-V5

In each X-Y contact map:

- region X (C3) aminoacids are indicated by the violet horizontal bar
- region Y (either V4 or V5) aminoacids are indicated by the pink vertical bar
Results constant region (I)

• C3 shows slight but *not significant changes of sequence length* in the different disease stages. C4 remains *constant always*.

• Conserved PNGS in V3 are mainly in the N-terminal residues, with *absence of shifting (variable) PNGS*

PNGS in C3 are spread out. **PNGS shifting is observed** in the N-terminal of C3 around the conserved PNGS 332 and 339 *where the alpha2 helix is localized*, and in C-terminal around the conserved PNGS N356 near the CD4 binding site.

**PNGS in C4 are concentrated** mainly in the middle portion of the region, between the conserved PNGS N442 and N448 near the CD4 binding site.

**All fixed PNGS in V3, C3 and C4 are also highly conserved** (≥ 70.8 %) in all variants and *in all disease stages*, with the only exception of the PNGS N339 in C3 which, in late infection, is *poorly conserved* (41.7 %).
Results constant regions (II)

• In V3 few aa residues are under selective pressure near the CCR5 coreceptor binding site, increasing in chronic and decreasing in late infection stage

• In C3 PS sites are generated during recent infection, increase in the chronic infection stage and decrease in late infection stage. They are located mainly inside the alpha2 helix. Few are near the CD4 binding site.

• In C4, both in recent and in chronic infection, the residue 429 is the only single amino acid under positive selection. In late infection two amino acids residues, 429 and 446, are found to be under positive selection, both near to the CD4 binding site.
Results constant regions (III)

• In the C3-V4 region a statistically significant inverse correlation in total charge during only the chronic infection, is seen.

• In the C3-V5 region a significant positive correlation in total charge for C3-V5 region in recent and late infection is found.

• C3 N-terminal α2-helix interacts strongly with V4 C-terminal (aa 412, 413, 414), across its positive selected residues 335, 346. In addition, the C3 region, with its C-terminal PS sites 360, 362, 364, strongly interacts with V4 N-terminal-located residues 391 to 395.

• C3 residue PS 360 strongly interacts with the V5 C-terminal-located residues 465 to 468.
Conclusions

Several mechanisms for virus escaping could be hypothesized:

• The increase of aa sequence length and shifting PNGS number at the C-terminal of V1 and V4 regions in chronic infection.
• PNGS shifting into the alpha2-helix in C3, around the conserved N356 and, in C4, N442 and N448, near the CD4 binding site, could have a role in protecting the virus from the immune response.
• The increase of PS sites in C3 and, particularly, in the α2-helix and in C-terminal, during CI stage. These PS could have a role in modifying Env conformational structure (as also hypothesized on the basis of contact map data).
• The presence of a negative correlation of positive electric charges between the C3 and V4 regions in the CI stage. This increases the electrostatic interaction between these two regions and could impact the Env conformation and glycan shield in order to protect the C3-V4 epitope.
• In a complementary way, the observed positive charge correlation between C3 and V5 in RI and LI stages could reinforce the hypothesis that during the early or late disease an open conformation could have the best fitness to bind cellular receptors.
Acknowledgments

Ndlela Research Centre
Eftyhia Vardas

NRL, Mbabane Government Hospital
Hosea Sukati

Department Technology and Health (ISS)
Giuseppe D’Avenio

Department Infectious Disease (ISS)
Massimo Ciccozzi
Alessandra Lo Presti

Retrovirus infection in developing countries
(CNAIDS-ISS)
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Pilar Narino
Simone Becattini
Michele Chiappi
Laura La Torre
Stefano Orrù

Clinical Trials (CNAIDS-ISS)
Orietta Picconi

Virus-host interaction(CNAIDS-ISS)
Paolo Monini

National AIDS Center- ISS
Barbara Ensoli

Presented at the 6th Int. Workshop on HIV Transmission, 14 – 15 July 2011, Rome, Italy