Human immunodeficiency virus type 1 intersubtype recombinants predominate in the AIDS epidemic in Cameroon

Judith N. Torimiro1,4, Roberta D’Arrigo3, Desire Takou1, Aubin Nanfack1, Daniele Pizzi3, Innocent Ngong1, Jean K. Carr5, Fouda Pierre Joseph1,4, Carlo-Federico Perno3, Giulia Cappelli1,2

1Centre International de Référence “Chantal Biya”, Yaounde, Cameroon; 2CNR, National Research Council, Rome, Italy; 3National Institute for Infectious Diseases “L. Spallanzani”, Rome, Italy; 4Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon; 5Institute of Human Virology, University of Maryland, Baltimore, USA

INTRODUCTION

The molecular epidemiology and genetic diversity of the human immunodeficiency virus type 1 (HIV-1) reflect an old infection in Cameroon, although the prevalence is low by African standards. Contemporary HIV-1 strains isolated from Cameroon in the early epidemic show a viral population of non recombinant forms compared to reports of recent studies. Complete genomes of HIV-1 subtype D (Kijak et al., 2004), subtype F2 (Peeters et al., 2000; Carr et al., 2001; Kijak et al., 2004) subtype K (Triques et al., 1999; Peeters et al., 2000), CRF02_AG (Kijak et al., 2004), CRF25_cpx (Luk et al., 2008; Carr JK, unpublished), CRF36_cpx (Powell et al., 2007), CRF37_cpx (Powell et al., 2007) and partial gag, pol and/or env sequences of subtypes A, B, C, D, F2, G, J and K and CRF06_cpx, CRF09_cpx, CRF11_cpx, CRF13-cpx (Triques et al., 1999; Peeters et al., 2000; Carr JK, et al., 2001, Fonjungo et al., 2002; Konings et al., 2004; Kijak et al., 2004; Ndongmo et al., 2006, Brennan et al., 2008) have been reported from Cameroon. The rare HIV-1 Group O (Gurtler et al., 1994; Ayoub et al., 2000; Yamaguchi et al., 2003; Bodelle et al., 2004; Brennan et al., 2008), HIV-1 Group N (Simon et al., 1998; Ayoub et al., 2000; Bodelle...
et al., 2004; Yamaguchi et al., 2006; Brennan et al., 2008), HIV Type 2 (HIV-2, Ndembí et al., 2007; Brennan et al., 2008), mixed infections A/J/U (Fonjungo et al., 2000), A/C, A/D/Group O, B/A (Takehisha et al., 1998), A/G/Group O (Peeters et al., 1999), and HIV-2 intergroup recombinant (Yamaguchi et al., 2008) have also been reported from Cameroon. Genetic variation of HIV-1 was recognized early in the epidemic and the challenges it presents to the development of diagnostic tests, antiretroviral (ARV) drugs, and AIDS vaccines were well appreciated. The questions that are not fully addressed are related to the factors that modulate a rapidly changing epidemic in the central African region. We describe the genetic diversity of HIV-1 from a population recently sampled to provide more information and an update of the variants co-circulating and that could be transmitted.

MATERIALS AND METHODS

Reverse transcription - Polymerase chain reaction and DNA Sequencing

Blood specimens were collected from HIV-positive individuals attending the “Chantal Biya” International Reference Centre (CIRCB) clinical services for routine follow-up from 2007 to 2008. Plasma RNA samples (extracted using the QiamRNA minikit, Qiagen) were genotyped using the ViroSeq HIV-1 Genotyping System v2.0 (Abbott Diagnostics). The RT-PCR steps amplify HIV-1 protease gene (from codons 1 to 99) and the reverse transcriptase gene (from codons 1 to 335) to generate a 1.8kb amplicon. Positive and negative controls were included in each RT-PCR reaction run. 1% agarose gel electrophoresis was used to confirm the amplification of the appropriately sized PCR product using a 100bp DNA ladder. This amplicon was sequenced using seven primers to generate continuous overlapping bidirectional sequences of about 434 codons of the HIV-1 protease and reverse transcriptase (RT) genes using the ABI Prism 3130 Genetic Analyzer (Applied Biosystem). The ViroSeq® HIV-1 Genotyping System software was used to assemble the sequence and compare it with the reference sequence, HXB2.

Analysis of sequence data sets

The DNA Sequence Analysis software version 5.2 (Applied Biosystems) was used for editing and the DNAMAN sequence analysis and alignment software to trim and resolve any ambiguities in the consensus sequences. Alignment was obtained with HIV-1 reference sequences followed by neighbour-joining performed with the Kimura two-parameter method of distance calculation and bootstrap analysis with 100 replicas. A Phylogenetic tree was constructed using MEGA3 software (http://www.megasoftware.net) for the sequences that were not unique recombinant forms (URF).

RESULTS

From 30 individuals (15 sampled in 2007 and the remaining in 2008), HIV-1 proRT sequences were genotyped. Of these, 13 (43%) were female and 9 (30%) were children below the age of ten years (Table 1).

<table>
<thead>
<tr>
<th>Code</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Year of specimen collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>795cirb</td>
<td>Female</td>
<td>9</td>
<td>2007</td>
</tr>
<tr>
<td>881cirb</td>
<td>Female</td>
<td>30</td>
<td>2007</td>
</tr>
<tr>
<td>931cirb</td>
<td>Male</td>
<td>not available</td>
<td>2007</td>
</tr>
<tr>
<td>940cirb</td>
<td>Male</td>
<td>43</td>
<td>2007</td>
</tr>
<tr>
<td>941cirb</td>
<td>Female</td>
<td>9</td>
<td>2007</td>
</tr>
<tr>
<td>961cirb</td>
<td>Female</td>
<td>49</td>
<td>2007</td>
</tr>
<tr>
<td>966cirb</td>
<td>Male</td>
<td>40</td>
<td>2007</td>
</tr>
<tr>
<td>992cirb</td>
<td>Male</td>
<td>5</td>
<td>2007</td>
</tr>
<tr>
<td>994cirb</td>
<td>Female</td>
<td>45</td>
<td>2007</td>
</tr>
<tr>
<td>1016cirb</td>
<td>Female</td>
<td>39</td>
<td>2007</td>
</tr>
<tr>
<td>1017cirb</td>
<td>Male</td>
<td>53</td>
<td>2007</td>
</tr>
<tr>
<td>1049cirb</td>
<td>Female</td>
<td>39</td>
<td>2007</td>
</tr>
<tr>
<td>1054cirb</td>
<td>Male</td>
<td>29</td>
<td>2007</td>
</tr>
<tr>
<td>1331cirb</td>
<td>Female</td>
<td>34</td>
<td>2007</td>
</tr>
<tr>
<td>1645cirb</td>
<td>Male</td>
<td>2</td>
<td>2007</td>
</tr>
</tbody>
</table>
Analysis of these 30 sequences showed that 26 (86.7%) were recombinant strains. Bootscan and phylogenetic analysis of 23 sequences that were not URFs were performed with reference sequences representing different HIV-1 clades (Fig. 1).

1) HIV-1 Circulating Recombinant Forms (CRF)

Of the 30 samples analysed, 20 (66.7%) were circulating recombinant forms (CRF) (Figure 1). Phylogenetic analysis of the 30 samples showed that 26 (86.7%) were recombinants of which 20 (66.7%) were Circulating Recombinant Forms (CRF). Of the 20 CRFs identified in this group of patients, 15 were CRF02_AG (50%), 1 of CRF06_cpx (3.3%), 3 of CRF11_cpx (10%) and 1 of CRF37_cpx (3.3%).

2) Unique Recombinant Forms (URF)

Six (20%) URFs were identified from this cohort of patients and their composition is shown in schematic diagrams below. The Recombinant HIV-1 Drawing tool was used to map the recombinant breakpoints for HIV-1 onto a map of the
FIGURE 2 - Maps of six Unique Recombinant Forms.
HXB2 genome. The different subtypes that compose the genome appear as differently-coloured regions in the map (Figure 2) (http://www.hiv.lanl.gov/content/sequence/HIV/refer.html). The mosaic composition of the 6 URFs consisted of segments derived from HIV-1 subtype A1, F2, J and K, CRF01_AE, CRF11_cpx and unclassified regions. Of these 6 URFs, 4 (66.7%) contained an unclassified region and 2 (33.3%) were second generation recombinants: URF(A1) and URF(U/11). Analysis of the recombination pattern and breakpoint distribution among these URFs, reveal that breakpoints occurred at nucleotide positions 2484 and 2529 (protease gene) in 2 of the URFs (F2/U - Figure 2b and F2/J - Figure 2e), and also at 3129, 2954 and 2754 (reverse transcriptase gene) in 3 (U/211 - Figure 2a, A1 - Figure 2d and K/U - Figure 2f).

3) HIV-1 subtypes
The phylogenetic analysis of the 23 proRT sequences showed that 4 (13.3%) were non recombinant variants of subtype A1 (n=1, 3.3%), C (n=1, 3.3%) and subtype D (n=2, 6.7%) (Figure 1). The HIV-1 subtype A1 is not shown in the phylogenetic tree because it showed 77% of A1 and the remaining sequence was hyper mutated.

DISCUSSION
Since the first cases of HIV infection were identified in Cameroon (Zekeng et al., 1990) several questions still linger about its evolution in the Central African region. In the early epidemic, a few subtypes were reported from Cameroon. As more samples from Cameroon were genotyped, a more statistically acceptable report on both the seroprevalence and molecular epidemiology of HIV was presented. Other pointers to the impact of the dynamics or rapidly changing epidemiology of HIV in Cameroon and the rest of the central African region were discordant results obtained from commonly used serological assays over the years. In the meantime, individuals became infected with two or more variants of HIV and/or SIV-like viruses (Peeters et al., 1994, Peeters et al., 2002, Van Heuverswyn et al., 2007) which may have gone undetected due in part to inappropriate screening algorithms.

During the era when the use of highly active anti-retroviral therapy in Cameroon was very low, the AIDS epidemic was driven by non recombinant subtypes A1, C, D, F, G, K and J and a few less complex recombinant variants. This epidemic changed to one predominated by CRF02_AG of up to 58.2% (Carr et al., 2001; Nyambi et al., 2002, Montavon et al., 2002, Tebit et al., 2002, Njai et al., 2006, Brennan et al., 2008). From samples collected in the late 1990s, new CRFs were identified: CRF06_cpx, CRF11_cpx, CRF13_cpx (Nyambi et al., 2004; Kijak et al., 2004; Ndongmo et al., 2006). In 1998, Simon and colleagues described the HIV-1 Group N viruses from a cameroonian woman which possesses segments from HIV-1 Group M and simian immunodeficiency virus (SIVcpz) from chimpanzee Pan troglodytes troglodytes isolated in Gabon (Simon et al., 1998).

Wolfe and colleagues have described zoonotic infections of simian foamy virus (SFV, Wolfe et al., 2004) and primate T lymphotropic virus (PTLV, Wolfe et al., 2005), from hunters in Cameroon. These facts also support Peeters and colleagues reports of the possible transmission of SIV from chimpanzees to humans in the central African region. Therefore, whether within humans or another reservoir, HIV-1, HIV-2 and SIV-like viruses show the propensity to recombine between groups, subtypes or circulating recombinant forms, as an immune escape mechanism. In this evolution process, new recombinant variants or forms of HIV-1 evolve suggesting that dual infection occurs frequently in this population.

Brennan et al. reported a mature AIDS epidemic in Cameroon after studying blood donor samples collected from 1994 to 2004 (Brennan et al., 2008). From rural hunting populations of low HIV prevalence (Torimiro et al., 2007), several recombinants have been reported (Nyambi et al., 2002) and recently, Yamaguchi (Yamaguchi et al., 2008) and Ndemb (Ndemi et al., 2008) also described HIV-2, HIV-1 and HIV-1 & HIV-2 intergroup recombinants in Cameroon. We identified one patient infected with CRF37_cpx, an old strain of genetically diverse lineages of HIV-1 subtypes, which others have also reported (Powell et al., 2007; Brennan et al., 2008). These reports indicate the broad diversity of HIV-1 in Cameroon and therefore the need for regular and systematic surveillance in this region.

From thirty patients attending the CIRCB clinical services, 86.7% were infected with a CRF or an
REFERENCES


ViroSeq HIV-1 Genotyping System v 2.0 (Abbott Diagnostics).


