Characterization of HIV Type 1 Clades in the Caribbean Using pol Gene Sequences

H. E. VAUGHAN,1 P. CANE,2 D. PILLAY,2,4 and R. S. TEDDER3

ABSTRACT

To date, 11 HIV-1 M group clades, A to K, have been characterized, displaying different distributions, prevalences, and biological properties. Approximately 90% of new HIV-1 infections occur in developing countries, including the Caribbean. However, information on HIV-1 subtypes from this region is limited. We report subtype characterization of viruses from 71 individuals, obtained during the period 2000–2002. RNA from the pol region was sequenced, generating data on subtype and drug resistance associated mutations for 71 specimens from 9 countries. Sixty-seven (94.4%) sequences were classified as clade B, three (4.2%) as D/B, and one (1.4%) as clade C. Numerous polymorphisms were observed, including some associated with drug resistance, but not signifying exposure to chemotherapy. This study adds to our knowledge of HIV-1 clades in the Caribbean, and indicates possibilities for monitoring HIV-1 chemotherapy.

Eleven subtypes of M-group HIV-1, A to K, have been described to date, and there is no apparent linear descent relationship between clades.1 They may have varying biological properties and display different geographical distributions and prevalences, necessitating a need for regional characterization and surveillance. In the absence of surveillance, the emergence of new clades in a region may result in an underestimate of the impact of the epidemic.

Approximately 90% of new HIV-1 infections occur in developing countries, including the Caribbean.2 However, information regarding circulating HIV-1 clades from this region is limited. Early studies reveal that in the Americas, including the United States, Brazil, Haiti, and Trinidad, clade B has predominated.3–5 Studies in several countries, including Cuba, Brazil, Venezuela, and Thailand,3,6–8 have found sudden shifts in subtype frequencies over just a few years, underlying the importance of assessing trends.

Early molecular studies used variability in env to determine HIV-1 clade, either by sequencing or heteroduplex mobility assay.6,7,9–12 More recently, pol has also shown sufficient variation to allow classification.13,14 For this study, pol was selected to allow both cladistic analysis and identification of drug resistance.

Antiretroviral therapy comprises two main classes of drug: targeting viral protease (PR) and viral reverse transcriptase (RT), often used in combination. Most resistance-conferring mutations occur in the pol gene, which constitutes the coding region for protease, reverse transcriptase, and integrase. Some commercial systems (e.g., ABI and Visible Genetics) have been developed to detect important mutations, and some reference laboratories, for example, in France and the United Kingdom, have developed in-house methodologies.13,15

The Caribbean Epidemiology Centre (CAREC) is a public health organization administered by the Pan American Health Organization (PAHO), WHO’s regional office for the Americas. CAREC provides reference laboratory services to 21 countries in the region.16 Consequently, CAREC has a role both in the surveillance of HIV and as a serum bank. The purpose of this study was primarily to identify which clades are currently circulating in the region and to allow evaluation of the tech-
ology by CAREC/PAHO/WHO for transfer and implementation within the Caribbean, potentially allowing the continuation of surveillance including monitoring of drug therapy.

Sera from the Caribbean Epidemiology Centre (CAREC) were used, representing individuals from 10 countries, naive to antiretroviral drugs. Specimens met the following essential criteria: (1) over 300 μl per sample, received at CAREC non-hemolysed, and stored frozen at −20°C; and (2) specimens received from 2000 to 2002 were HIV positive by two different enzyme immunoassays (EIAs) (two-step sandwich EIA to detect IgM, IgG, and IgA and one-step sandwich EIA to detect both antibody and antigen simultaneously).

Ten countries were represented by the 94 samples (Table 1): Antigua (10), Dominica (5), Grenada (4), Guyana (12), Montserrat (1), St. Lucia (7), St. Vincent (7), St. Kitts (1), Suriname (7), and Trinidad and Tobago (40), with 34 males, 54 females, and 6 of unknown gender. Ages ranged between 2 and 65 years, with a mean, median, and mode of 22 years.

RNA was extracted from serum specimens using the Qiagen Biorobot 9604 using an input of 200 μl and elution volume of 60 μl. Positive control was plasma containing 500,000 copies HIV RNA/ml. Negative control was uninfected plasma.

Reverse transcription polymerase chain reaction (RT-PCR) and sequencing were carried out as previously described, except that a one-step RT-PCR protocol (Qiagen) was used.

Sequences were aligned and edited using Sequencher (Gene Codes Corporation). The Stanford University website (http://hivdb.stanford.edu/hiv) was used for subtype determination. Information on drug resistance was also provided at this site.

Seventy-one of 94 specimens yielded between 1022 and 1200 bp contiguous, overlapping sequence in both directions, comprising both protease and the upstream region of the RT gene. For eight nonamplifiable samples, for which sufficient sample was available, HIV infection was confirmed using the Fujirebio gelatin agglutination antibody test. Failure to amplify product for these samples was likely due to low viral load or non-subtype B subtype. A neighbor-joining tree was constructed using the sequence alignment generated plus reference sequences of clades B, C, and D, respectively, registered in GenBank. The neighbor-joining tree was obtained using PAUP 4.0 (Fig. 1), after using Modeltest 3.06 PPC to choose the most appropriate model. Subtype designation of specimens was compared with those generated from the NCBI subtyping site (http://www.ncbi.nlm.nih.gov/retroviruses/subtype/subtype.html).

Sixty-seven specimens (94%) were classified as clade B using the Stanford system. Four specimens were classified as potentially non-B; of these, one (1.4%) was C with respect to both protease and RT, ID #133, and three (4.2%) were D/B, i.e., B with respect to RT and D with respect to protease sequence (ID #26, #38, and #113). Using the NCBI system and reference strains the majority of specimens were clade B (98.6%). Many of these had regions of similarity to clade D (40%), and some showed regions of similarity to other clades, specifically C, F, and G (7%). The large proportion of specimens with D-like stretches could be a result of the underrepresentation of Caribbean strains in the NCBI B clade reference database.

However, clades B and D have been shown to be closely related with respect to gag, env, and pol sequences and probably diverged relatively recently. Thus separation of these lineages is not as well defined as between other clades. The neighbor-joining tree generated after modeling grouped these three D/B specimens with B clade viruses (Fig. 1). Future studies, including those with an epidemiological component, could shed light on the distribution and prevalence and possibly result in more accurate classification of HIV-1 viruses, and indeed, other aspects of HIV spread in the region.

No primary drug resistance-associated mutations were detected in these samples. Some secondary mutations were identified in some specimens. However, these were exclusively natural polymorphisms that can be associated with drug resistance when present with other mutations, but do not in themselves signify problems with or previous exposure to chemotherapy. This confirms expectations that the population sampled has not been exposed to antiretroviral therapy. While it is reassuring...

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of isolates</th>
<th>Number producing sequence for PR and RT</th>
<th>Clades represented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigua</td>
<td>10</td>
<td>8</td>
<td>B, B/D</td>
</tr>
<tr>
<td>Dominica</td>
<td>5</td>
<td>5</td>
<td>B</td>
</tr>
<tr>
<td>Grenada</td>
<td>4</td>
<td>4</td>
<td>B</td>
</tr>
<tr>
<td>Guyana</td>
<td>12</td>
<td>6</td>
<td>B</td>
</tr>
<tr>
<td>Montserrat</td>
<td>1</td>
<td>1</td>
<td>B</td>
</tr>
<tr>
<td>St. Kitts</td>
<td>1</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>St. Lucia</td>
<td>7</td>
<td>5</td>
<td>B, C</td>
</tr>
<tr>
<td>St. Vincent</td>
<td>7</td>
<td>6</td>
<td>B</td>
</tr>
<tr>
<td>Suriname</td>
<td>7</td>
<td>5</td>
<td>B</td>
</tr>
<tr>
<td>Trinidad and Tobago</td>
<td>40</td>
<td>31</td>
<td>B, B/D</td>
</tr>
</tbody>
</table>

aOverview of cladistic analysis results by country.
that circulating viruses do not appear to be drug resistant, the findings highlight the need for effective therapeutic management of patients in the Caribbean.

In summary, clade B viruses are still the predominant circulating virus in the sample analyzed. However, the presence of additional clades is notable, especially given the relatively small sample size. A single clade C virus was identified, from St. Lucia, 2002. This clade is fairly widespread in sub-Saharan Africa. Ongoing surveillance will be useful to continue to monitor the epidemic.

Accession numbers are K033455 subtype B (HXB2), US2953 subtype C (92BR025C), and K03454 subtype D (ELID).

FIG. 1. Neighbor-joining tree including all specimens sequenced plus reference sequences for B, C, and D clades. Specimens are indicated by number. Reference sequences, denoted HXB2short (clade B), 92BR025C (clade C), and ELID (clade D), represent equivalent regions of pol in the reference sequences used. See text for details.

ACKNOWLEDGMENTS

This project was funded by the Special Programme on Sexually Transmitted Infections (SPSTI), CAREC/PAHO/WHO, and the authors would like to thank the Programme and its contributors, as well as the London School of Tropical Medicine and Hygiene, which also contributed. The authors are indebted to the staff of the Virology Unit, Windeyer Institute, UCL, especially Paul Grant, David Bibby, and Julie Bennett, the Windeyer Institute Sequencing Service, and PHLS Birmingham, especially Ms. Daina Ratcliffe. We are also grateful to Cat Gale and Paul Kellam of the Wohl Virion Centre, UCL, for helpful discussions and to Jerome Foster from the University...
of the West Indies Mount Hope for construction of phylogenetic trees.

REFERENCES


Address reprint requests to:

H. E. Vaughan
Caribbean Epidemiology Centre (CAREC) 16-18 Jamaica Boulevard Federation Park, Trinidad and Tobago
This article has been cited by:

1. Lissette Pérez, Michael M. Thomson, María J. Bleda, Carlos Aragonés, Zoila González, Jorge Pérez, María Sierra, Gema Casado, Elena Delgado, Rafael Nájera. 2006. HIV Type 1 Molecular Epidemiology in Cuba: High Genetic Diversity, Frequent Mosaicism, and Recent Expansion of BG Intersubtype Recombinant Forms. *AIDS Research and Human Retroviruses* **22**:8, 724-733. [Abstract] [PDF] [PDF Plus]