Molecular Characterization of the Human Immunodeficiency Virus Type 1 among Children in Lima, Peru

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Abstract

In Peru, there is a lack of information on molecular analysis in pediatric human immunodeficiency virus (HIV) infection. At present, the mother-to-child transmission rate is estimated at approximately 2–4%. The objective of this study was to assess the molecular epidemiology of HIV-1 in infected children. Children with suspected or confirmed pulmonary tuberculosis were evaluated at two public hospitals between 2002 and 2007. Whole blood samples were obtained from 90 HIV-positive children, who were confirmed to be positive by enzyme-linked immunosorbent assay and Western blot. The specimens were subjected to envelope heteroduplex mobility assay (env HMA) followed by *gag* and *pol* gene region sequence analysis. Subtype B was found in 88 (98%) of 90 children and 2 (2%) children were subtype BF recombinants. This is the first report of recombinant HIV strains in HIV-infected children in Peru. Understanding the origin, diversity, and spread of HIV strains worldwide will be necessary for the development of an effective vaccine that targets pediatric populations throughout the world.

Introduction

HTRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS type 1 (HIV-1) from an infected mother to her child can occur *in utero*, during birth, or postnatally through breast milk, which is the main source of pediatric HIV-1 infection. The prevalence of HIV-1 infection among women and children is increasing at an alarming rate worldwide. Mother-to-child transmission of HIV-1 remains a common route of infection despite the introduction of short-course peripartum antiretroviral therapies.¹ Approximately 630,000 children are infected yearly, most of them born in developing countries.² In Peru, it is estimated that vertical transmission accounts for 2–4% of all HIV-infected cases.³

We describe the molecular characterization of HIV-1 in Peruvian children.

Materials and Methods

We sampled children between the ages of 6 months and 8 years at two large public hospitals over a period of 5 years

(October 2002–February 2007) in Lima, Peru under the protocol entitled “Practical Diagnostics for AIDS-Related Pediatric TB, Peru” (NMRC.D.2002.0014) performed to evaluate the utility of new diagnostic approaches for TB in children in resource-poor countries. A clinical trial was conducted in Lima, Peru among children without evidence of HIV infection and among children with confirmed HIV infection. NMRC.D genotyped HIV-positive samples and molecular characterization of those samples are described in this article.

Only children who were suspected of having or confirmed as having pulmonary tuberculosis (Tb) were enrolled. HIV infection was initially detected by screening of serum samples by enzyme-linked immunosorbent assay (EIA) and then confirmed by Western blot (WB) testing. Proviral DNA was extracted from peripheral blood mononuclear cells (PBMCs) with the QIAamp blood kit (Qiagen GmbH, Hilden, Germany).

Samples were subjected to polymerase chain reaction (PCR) amplification in the envelope (*env*) region and *env* sequence subtypes were determined by heteroduplex mobility analysis (HMA) using prototypic subtypes as previously

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TABLE 1. HIV SUBTYPE BY HETERODUPLEX MOBILITY ASSAY, GAG, AND POLYMERASE/REVERSE TRANSCRIPTASE GENE REGION AMONG HIV-POSITIVE CHILDREN^a

Subtypes	<i>env</i>		<i>gag</i>		<i>pol</i>
	HMA	Sequencing	HMA	Sequencing	Sequencing
B	88	16	19	87	—
F	2	2	N/D	2	—
BF	—	—	—	—	2
Total	90	18	19	89	2

^aHMA, heteroduplex mobility assay; *env*, envelope gene region; *gag*, gene region; *pol*, protease/reverse transcriptase (Pro/RT) gene region; N/D, not determined.

described.⁴ Additionally, the *gag* (p24) and *pol* (pro/RT) gene regions were sequenced to further characterize potential recombinant viruses. Direct sequencing of both sense and antisense strands was performed with the Big Dye Terminator kit (Applied Biosystems, Foster City, CA). Sequencing reaction products were analyzed on a 3130X-Genetic Analyzer automated sequencer by using the inner amplification primers (Applied Biosystems). Analyses of HIV-1 subtypes and recombination were performed using the Recombinant identification Program (RIP) HIV-1 Subtyping tool (Los Alamos HIV Database).

Sequences were deposited in GenBank under accession numbers FJ744747 and FJ744748.

Results

During the study period, serum samples were obtained from 682 children with suspected or confirmed pulmonary

tuberculosis. Of these, whole blood samples were obtained from 116 HIV-positive children: mean (SD), 4.5 (2.9 years); median (range), 4.1 (3 months to 10.5 years). Genotypic characterization by HMA was possible in 90 (77.5%) out of 116 samples. Eighty-eight (98%) were subtype B and 2 (2%) were subtype BF recombinant forms (Table 1) based on the sequences of the *gag* and *pol* gene regions. Figure 1 shows the recombinant regions of these strains using the complete genome of HXB2 as the reference strain.

Discussion

This study reports, for the first time in Peru, the molecular characterization of the HIV-1 viral strains circulating among children. Results related to new diagnostic approaches for pediatric tuberculosis among Peruvian children with and without HIV coinfection will be given in a different publication.

In Peru, HIV screening of pregnant women is not mandatory. Peruvian law states that it is voluntary and women must sign a consent form. HIV screening is available in the MoH Health Centers where HAART is also available. For all Peruvians with pulmonary tuberculosis free treatment is given by the Ministry of Health for 6 months with four drugs.⁵

Subtype BF recombinants were found in two samples. This represents evidence of the continuing evolution of HIV-1 infection in this country. In South America, subtype B is still the major genetic form circulating, with a small proportion of other subtypes such as F, BF, and other circulating recombinant forms (CRFs)^{6–8} such as CRF12_BF found to be prevalent in heterosexual groups and among vertically infected children in Argentina, Uruguay, and Brazil.^{9–14}

In Brazil, new BF subtype CRFs have been previously identified. These recombinants, named CRF28_BF, CRF29_BF,

FIG. 1. Diagram based on the HXB2 complete genome showing the recombinant regions of the HIV strains DAM 1245 and DAM 1286. The figure was made using the jumping profile hidden Markov model (jpHMM) downloaded from the Los Alamos database.

CRF39_BF, and CRF40_BF, are the new CRFs composed exclusively by subtypes B and F,¹⁵ and CRF38_BF encountered in Uruguay (unpublished). Phylogenetic analyses based on the *gag-pol* region show that the identified recombinants from Peru (DAM 1245 and DAM 1286) are related genetically to the HIV recombinants from Brazil and Argentina (data not shown). The combination of *gag* and *pol* gene subtyping results indicates the presence of different subtypes in our HIV-infected population of children. Thus, genotyping based on the *pol* gene region may show that samples are recombinants, and could be used as a rapid approach for monitoring the prevalence of HIV-1 recombinants. Future studies are necessary to fully characterize genetically these BF recombinant strains from Peru.

In summary, we documented molecular genotyping evidence of subtype BF recombination forms among HIV-positive Peruvian children. Additional studies among HIV-infected children are required to fully understand the molecular epidemiology of infection in this country and in the Andean region. The data presented here contribute to our understanding of the diversity and spread of HIV strains, as well as the emergence of subtypes, CRFs, and intrasubtypes that may be more readily transmitted or have altered virulence.

Acknowledgments

This work was funded under number 62787A. S17.H. B0002. The views expressed in this article are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy or Army, Department of Defense, or the U.S. Government. The study protocol was approved by the Naval Medical Research Center Institutional Review Board (Protocol #NMRCD.2002.0014, DoD #31599, WRAIR #966) in compliance with all applicable Federal regulations governing the protection of human subjects.

Some of the authors are military service members and employees of the U.S. Government. This work was prepared as part of their official duties. Title 17 U.S.C. §105 provides that "Copyright protection under this title is not available for any work of the United States Government." Title 17 U.S.C. §101 defines U.S. Government work as work prepared by a military service member or employee of the U.S. Government as part of that person's official duties.

Disclosure Statement

No competing financial interests exist.

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