SHORT REPORT: MOLECULAR EPIDEMIOLOGY OF HUMAN IMMUNODEFICIENCY VIRUS– INFECTED INDIVIDUALS IN MEDELLIN, COLOMBIA

GLORIA I. SANCHEZ, CHRISTIAN T. BAUTISTA, LINDSAY EYZAGUIRRE, GLADYS CARRION, SONIA ARIAS, WARREN B. SATEREN, MONICA NEGRETE, SILVIA M. MONTANO, JOSE L. SANCHEZ, AND JEAN K. CARR*
Grupo Infección y Cáncer, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia; United States Military HIV Research Program at the Walter Reed Army Institute of Research, and the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Rockville, Maryland; Sección de Enfermedades Infectiosas, Departamento de Medicina Interna, Hospital Universitario, San Vicente de Paul, Medellín, Colombia; United States Naval Medical Research Center Detachment, Lima, Peru

Abstract. To study the molecular epidemiology of human immunodeficiency virus type 1 (HIV-1) strains in Medellín, Colombia, 115 HIV-1–positive individuals who were recruited from an HIV outpatient hospital (Universitario San Vicente de Paul) during the period from July 2001 to January 2002 were genotyped. All samples were analyzed by envelope heteroduplex mobility assay and found to be subtype B. Twenty-four samples were randomly selected for sequencing of the protease and the reverse transcriptase regions; all isolates were found to be subtype B. Phylogenetic analysis of seven nearly full-length genomes showed that all samples were subtype B. This study shows that the HIV epidemic in Colombia continues to be dominated by the subtype B virus. The predominance of subtype B genotypes of HIV-1 strains in Medellin resembles what is seen in the nearby countries of Peru, Ecuador, and Venezuela.

Previous molecular surveillance studies have showed significant heterogeneity in the prevalence and geographic distribution of human immunodeficiency virus type 1 (HIV-1) subtypes in South America. Approximately 80% of HIV infections have been reported to be envelope (env) subtype B, and 20% env subtype F, most of which were BF recombinants. Three subtypes (C, A, and D)2–4 and two circulating recombinant forms (CRFs)5–7 have been also identified in this region. Additionally, multiregion hybridization assay testing for subtypes B or F in countries of the Southern Cone region (Argentina, Uruguay, Paraguay, and Chile) of South America, where injecting drug use (IDU)–associated transmission occurs, showed predominantly BF recombinants.1,7 No systematic efforts have been made to characterize the subtype diversity of HIV-1 in Colombia.

The study was conducted in Medellín (State of Antioquia), the second largest city in Colombia. As of 2000, it had 6,692 reported HIV infections.8 We investigated the genetic diversity and epidemiology of HIV-1 among 115 HIV-infected patients seen from July 2001 to January 2002 at the Hospital Universitario San Vicente de Paul. The study protocol was reviewed and approved by University of Antioquia, U.S. Naval Medical Research Center Institutional Review Boards (Work Unit No. 62787A 873 H B0002), and the Walter Reed Army Institute of Research (WRAIR # 910) in compliance with all federal regulations governing the protection of human subjects.

All individuals were at least 18 years of age, provided written informed consent, socioepidemiologic data, and a blood sample. Confidential one-to-one interviews were conducted in a private room by a trained nurse with experience in prevention of HIV and sexually transmitted infections. Peripheral blood mononuclear cells (PBMCs) were isolated by the Ficoll gradient method (lymphocyte separation medium; ICN/Cappel, Aurora, OH) and DNA was extracted from approximately 1 × 10⁶ infected PBMCs by the QIAmp DNA extraction technique (Qiagen, Valencia, CA). A random sample of study participants was selected for genotypic analysis. DNA samples were genotyped using the envelope the env heteroduplex mobility assay (HMA) as previously described.3,9 Briefly, after two rounds of polymerase chain reaction (PCR) with primers ED14 and ED3 (first round) and ED12 and ED5 primers (second round), HMA electrophoresis was performed with second-round PCR products using nine reference standards in the formation of heteroduplex.

Amplification of the protease (Pro), and reverse transcriptase (RT) from PBMC DNA was performed on randomly selected samples from the sample set using a nested strategy. The first round amplification was done: Pro5F (5′-AGAAATTGCAGGGCCCCTAGGAA-3′) and RT3474R (5′-GAATCTCTCTTGTGTTCTGCGCA-3′) and AmpliTaq Gold (Applied Biosystems, Foster City, CA). Using 1 μL of product from the first round, the second round was completed using the following two primers: Pro3F (5′-AGAAATTGCAGGGCCCCTAGGAA-3′) and RT3474R (5′-GAATCTCTCTTGTGTTCTGCGCA-3′) and ProRT (5′-TCTTCCCTCAACTTCTGATGATGATGGA-3′). This nested strategy amplified 1.1 kb of the HIV genome. The amplified product was prepared for automated sequencing by purification in a YM-50 column (Amicon, Beverly, MA). The purified product was sequenced using fluorescent dye terminators with an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA). The region sequenced included all of protease and the carboxy terminus of the reverse transcriptase (RT) protein. Nearly full-length genomes were also amplified directly from PBMCs using a nested PCR strategy that amplified the region from gag to the end of the R region of the 3′ long terminal repeat.10

Sequences were assembled using Sequencer software (Gene Codes, Inc., Ann Arbor, MI) in a multiple alignment with standard subtype references. Phylogenetic analyses were conducted using neighbor-joining with Kimura’s two-parameter model of distance calculation and a bootstrap computed using maximum parsimony.11,12 This technique was used to create phylogenetic trees that were used to assign subtypes. Boot scanning and distance scanning were conducted to determine the presence of recombination by using...
A total of 248 HIV-1-infected individuals were enrolled (Table 1). Most study participants were male (84%), and there was a male:female ratio of 5:1, which is similar to the distribution of HIV-1-infected persons in Colombia. Almost half of the subjects were 34 years of age or less (range = 18–74 years). Only 12% of the study participants reported education at the college level or higher. Sixty-three percent of the participants reported Medellín as their primary residence, and 53% were employed although more engaged in informal commercial activities. The principal route reported for HIV infection was sexual (84% of males and 88% of females). Among men who reported a sexual route of HIV transmission, 47% reported having sex with men, 29% with women, and 44% with both sexes. By comparison, among the women, most (97%) reported having sex only with men and only two reported bisexual behavior. Approximately 20% of the study subjects reported sexual contact with foreigners from the United States (10%), Spain (4%), Venezuela (4%), Germany (3%), and Panama (2%). A report of HIV/acquired immunodeficiency syndrome for 2000 in the State of Antioquia noted that the HIV epidemic involves mainly young adults, and the second mode of HIV transmission in Colombia is heterosexual transmission, which is similar to that observed in our study population.

Of the 248 samples, 115 (46%) were selected at random for characterization of HIV-1 subtypes by HMA (Table 1). All selected samples were classified as 

\[ env \] subtype B. Partial Pro/RT sequencing was performed on a random subset of 24 samples; the phylogenetic analysis is shown in Figure 1A. This analysis showed that all samples were subtype B, and there was no evidence for a unique Colombian founder in our study population; the Colombian subtype B was similar to that observed previously in the United States, Europe, and the rest of South America. Nearly full-length sequencing and analysis of seven strains showed that all were subtype B. The nearly full analysis is shown in Figure 1B. A distance scan of the genome confirmed that each of the strains was subtype B throughout the genome, without signs of inter-subtype recombination.

To our knowledge, this is the first molecular epidemiologic study to document the distribution of HIV-1 genetic subtypes among HIV-infected individuals in Medellín, Colombia. The HIV epidemic in Medellín, as in the rest of Colombia, is dominated by sexual transmission among young men and women of low socioeconomic status (Sanchez GI, unpublished data). The predominance of a sexual route of transmission in our patient population is in agreement with what has been reported from other countries in the Andean region (Bolivia, Ecuador, Peru, and Venezuela) where approximately half (48%) of HIV cases have been diagnosed among men who have sex with men (MSM). In Colombia, MSM and heterosexual transmission constitute the main modes of HIV infection. IDU has been associated with low risk for HIV infection in this country. However, the HIV epidemic is slowly expanding among women, with an increasing role of homosexual/bisexual men serving as a key bridging population. Previously published studies have documented a high percentage (46%) of MSM who also have sex with women in the northern coastal city of Cartagena, as well as high-risk practices among men, particularly among heterosexuals in the capital city of Bogotá.

In 1993–1995, the first report on HIV-1 strains circulating in Colombia indicated that all of 12 sequences were subtype B. Another study, which included 4 HIV-infected MSM and 130 HIV-infected female commercial sex workers in Bogotá, also showed that subtype B strains were the most prevalent. In our study population, all HIV strains were subtype B. There was also no evidence for inter-subtype recombination in any of the full-length genomes sequenced.

The predominance of subtype B genotypes of HIV-1 in Medellín resembles what is seen in the nearby countries of Peru, Ecuador and Venezuela. However, in Argentina and Uruguay, it has recently become apparent that BF recombinants are predominate among infected heterosexuals (female homosexuals and their male partners), whereas subtype B predominated among MSM. In addition, the first report of the existence of a circulating recombinant form (CRF02_AG) in South America was found in a study conducted among HIV-positive patients in Guayaquil, the main port of Ecuador, approximately 1,000 km south of Medellín, and which is frequently visited by travelers from different parts of the world. The contribution of travel to the spread of HIV-1 around the world has been well documented. Travel-associated infections are mainly caused by non-B subtypes and CRFs and contribute to an increase in HIV-1 diversity, as shown in recent years in Asia, Europe, Brazil and Cuba. Since different HIV subtypes can occur in different populations and such diversity may influence therapeutic and vac-
A) Partial Pro/RT sequencing

B) Nearly full-length genome

Figure 1. A, Phylogenetic analysis of 24 partial protease/reverse transcriptase (Pro/RT) sequences of human immunodeficiency virus type 1 (HIV-1) from Medellín, Colombia (bold). A neighbor-joining analysis of the strains was done in conjunction with reference strains from the major subtypes using a parsimony bootstrap value for the node joining the Colombian strains with reference subtypes B and F. B, Phylogenetic analysis of 7 nearly full-length genome of HIV from Medellín, Colombia (bold). Analysis was performed using neighbor-joining with a bootstrap value computed using maximum parsimony. The scale bar below the tree shows a genetic distance of 10%.


