Presence of Human Immunodeficiency Virus (HIV) Type 1, Group M, Non-B Subtypes, Bronx, New York: A Sentinel Site for Monitoring HIV Genetic Diversity in the United States

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In the United States, human immunodeficiency virus (HIV) type 1, group M, subtype B is the predominant subtype. A cross-sectional study of HIV-infected patients at the Bronx-Lebanon Hospital Center, Bronx, NY, between September 1997 and February 1998 identified 3 (1.2%) of 252 persons infected with non-B subtypes: subtypes A and F, 1 each, and 1 potential recombinant subtype B(Env)/F(Prt). All 3 persons were born in the United States and tested positive for HIV antibodies between 1988 and 1997 while living in the Bronx. None reported travel to other countries, receipt of blood products, or drug injection. This study is among the first to indicate probable transmission of non-B HIV-1 subtypes in the United States. The occurrence of non-B HIV-1 subtypes in long-term US residents without a history of foreign travel may have implications for the evaluation and development of antiretroviral drugs, vaccines, and tests intended for use in the United States to diagnose HIV infection and screen blood.

Two main types of human immunodeficiency virus (HIV) have been characterized: type 1, the predominant HIV type worldwide, and type 2, which is still localized mainly in persons from West Africa [1]. Group M is the predominant group of HIV-1 and consists of 10 subtypes (A–J) based on genetic characterization of envelope regions. Groups O and N are less common. To date, most HIV isolates identified in North American residents are HIV-1, group M, subtype B, and most US residents infected with non-B subtypes acquired their infections abroad [2–5]. Indigenous transmission of non-B subtypes may occur in the United States [5–7], but the extent of such indigenous transmission has not been studied.

Bronx-Lebanon Hospital Center (BLHC) is the primary provider of health care to 450,000 South Bronx residents. It is located in an impoverished community where the prevalence of HIV and HIV risk behaviors (notably injection drug use and high-risk heterosexual sex) is among the highest in the United States. The community includes many recent immigrants and long-term foreign visitors from Latin America, the Caribbean, Africa, and Asia. Many residents travel outside the United States to visit family.

A nonmasked HIV serosurvey conducted at BLHC during 1992–1994 found that 5.2% of 828 inpatients and outpatients aged 18–44 years who had not reported HIV infection were HIV positive. Of the 43 patients confirmed to have newly identified infections who were subtype-d, 2 (5%) had subtype A infections, one of whom reported no foreign travel or birthplace [7].

Our objectives in this study were to evaluate the presence of non-B HIV-1 subtypes among a select group of HIV-infected persons who had resided in the United States since they were 15 years old, to identify HIV risk behaviors of persons associated with B and non-B subtypes, and to determine location of probable infection.

Materials and Methods

We recruited HIV-infected patients from the BLHC infectious disease clinic and inpatient services. The clinic serves >2000 patients...
with all stages of HIV infection and provides comprehensive, long-term primary and specialty medical and social services. Eligible patients (1) had a date of their first positive HIV antibody test in the medical record; (2) were ≥ 18 years old; (3) were not incarcerated or on parole; (4) had lived in the United States since age 15 years; (5) were mentally and physically able to provide informed consent; and (6) understood spoken English, Spanish, French, or one of three West African languages (Twi, Ga, or Hausa). We included only persons who had lived in the United States since age 15 years because we wanted to evaluate persons who most likely acquired HIV infection in the continental United States.

We attempted to recruit a total of 300 persons, with an equal number of men and women in each of 6 equally sized strata defined by time since first positive HIV antibody test (≤ 1 year, > 1 year through 4 years, > 5 years) and by sexual or injection drug contact with foreign nationals or persons who have lived or traveled outside the United States (yes, no). For this study, we defined "foreign contact" as sex or having shared injection equipment with a person who had traveled or lived outside the 50 US states or with a foreign national while traveling outside the 50 US states. We classified Puerto Rico as outside the 50 US states because of the substantial prevalence of HIV-1, group M, non-B subtypes [8].

Interviewers questioned patients about demographics, HIV risk characteristics and history of HIV disease, travel and residence, sexual activity, and injection of drugs. The interviewer also asked patients about travel and prior residences of their sex partners and persons with whom they shared injection drug equipment. After the interview, the interviewer drew blood, which was sent to the Centers for Disease Control and Prevention (CDC) for HIV subtype testing.

We serotyped all specimens by using investigational EIAs, including peptides representing HIV-1, group M, subtype V3 loop immunogenic domains of subtypes A–F and HIV-2, as described elsewhere [9]. Specimens that were reactive only to the V3-B peptides were considered subtype B. Laboratory personnel used standard methods to genetically sequence all specimens that were non-reactive to these V3-B peptides from proviral DNA from peripheral blood mononuclear cells [10, 11].

For genotypic characterization, we used gp41 sequence–based phylogenetic analysis for the 3' end and subtyping based on restriction fragment length polymorphism (RFLP) analysis of the protease gene (prt) for the 5' end of the genome. Our lab previously established that V3 sequencing–based subtypes are similar to those done by gp41 phylogenetic analysis [12, 13]. The primers and amplification conditions for protease and gp41 regions have been described elsewhere [10, 11]. To analyze potential recombinants, we analyzed prt by use of RFLP, as follows [10]. The restriction enzyme digestions consisted of 8 μL of nested prt, 5–10 U of AluI restriction enzyme, and 1 μL of the enzyme buffer supplied by the manufacturer. After overnight incubation at 37°C, the entire restriction digest reaction was electrophoresed in a 10% polyacrylamide gel and visualized by ethidium bromide staining. Positive controls representing distinct HIV-1 subtypes were included for the restriction enzyme digest to confirm the activity of the restriction enzyme and to provide expected restriction fragment patterns. The viral prt of specimens that seemed to be non–subtype B by RFLP assay was sequenced. Double-stranded viral DNA from gp41 amplified by polymerase chain reaction (PCR) [11] and selected protease gene products were cycle sequenced in both directions with fluorescent dye–labeled sequencing terminators [14]. Sequencing reactions were run in an automated DNA sequencer (Applied Biosystems, Foster City, CA). Aligned sequences, after the elimination of regions that contained gaps, were analyzed by maximum likelihood method by the fastDNAmI program [15] and by the neighbor-joining method [16]. Simian immunodeficiency virus sequences (SIVcpz; GenBank accession no. X52154) were used as outgroups.

Results

Patient characteristics. From September 1997 through February 1998, we evaluated 426 patients for study eligibility, of whom we enrolled 256 (49% women, 62% black, 35% Hispanic; table 1). Eighty-seven persons did not meet eligibility criteria, and 46 persons were eligible but were not needed in the sampling strata that remained unfulfilled. Of those eligible and needed for the sample requirements, 26 refused and 11 did not return for interview. Of the 256 persons in the study, 230 (90%) were enrolled from the infectious disease clinic and 26 (10%) from the inpatient services. The time between the first positive
Figure 1. Phylogenetic classification of human immunodeficiency virus type 1 (HIV-1) strains from persons in Bronx, New York, is denoted with BX prefix. Trees were constructed on the basis of DNA sequences of gp41 (a) and prt (b) (GenBank accession nos. AF1900947–AF190078) by the neighbor-joining method. Nos. at branch nodes connected with subtypes are bootstrap values. Three major branches within subtype F protease tree are marked by Brazilian (BR22, 7944), Puerto Rican (PR185), and Romanian (RO557, RO594) sequences. ●, Bronx samples that clustered into different subtypes depending on parts of genome analyzed; *, subtype A infections; ●, subtype F infection. Scale bar indicates evolutionary distance of 0.10 nt per position in sequence. Vertical distances are for clarity only.
HIV test result and enrollment was ≤1 year for 56 (22%), 1–4 years for 100 (39%), and ≥5 years for 100 (39%).

HIV risk behaviors. All subjects reported having had sex without a condom with ≥1 other person before HIV infection was diagnosed. All 126 women (100%) reported vaginal or anal sex with a man. Of 130 men, 125 (96%) reported vaginal or anal sex with a woman, and 45 (35%) reported having anal sex with a man. Injection drug use before HIV infection was known was reported by 102 (40%) of the 256 subjects. Of these, 81 (79%) reported sharing injection equipment. Fifty (20%) reported receiving a total of 51 blood transfusions before they knew they were HIV positive. Of the transfusions, 49 (96%) took place in the United States, 1 (2%) in Puerto Rico, and 1 (2%) at an unknown location. Nine persons (4%) reported receiving an injection from a health care provider outside the United States before HIV infection was diagnosed (6 in Puerto Rico and 1 each in Japan, Korea, and Mexico).

Needle sharing or sexual contact with foreign nationals or foreign travelers. Sexual contact or injection drug contact with a foreign national or foreign traveler before HIV infection was known was reported by 137 persons (54%). Contact included sex without a condom for 108 persons (79%), sharing of injection drug equipment by 4 persons (3%), and both for 25 persons (18%). Of the 133 subjects who reported sexual contacts with a foreign national or someone who had traveled outside the United States, 106 (80%) reported having such contacts in the United States and 5 (4%) while traveling outside the United States, and 22 (17%) reported both. Of the 29 persons who shared injection drug equipment with a foreign national or someone who had traveled outside the United States, 24 (83%) reported that the contact took place in the United States and 3 (10%) while traveling outside the United States, and 2 (7%) reported both.

Subtypes. Of the 256 specimens, 195 (76%) were found by peptide serology to be HIV-1, group M, subtype B, 1 specimen was repeatedly negative for HIV-1 and HIV-2 by conventional serology, and 60 specimens (23%) had serotypes that were not consistent with subtype B. These 60 specimens were chosen for genetic subtyping. Of these, 57 were PCR positive in the gp41 region, and 3 were PCR negative for the gp41 and protease regions of HIV-1 and for the HIV-2 protease (prt) gene, despite use of multiple primer pairs. Of the 57 PCR-positive specimens, 54 represented homologous infection with subtype B both in prt and in gp41 (figure 1). Thus, of 252 specimens that could be evaluated, 249 (98.8%) were group M, subtype B. Three (1.2%) specimens were non-B subtypes. One was subtype A by sequencing in the gp41 and protease regions. The second was negative by PCR amplification in the gp41 region, but gene sequencing confirmed that it was subtype F in the protease region. The third was a potential recombinant characterized by subtype B in the gp41 region and subtype F in the protease region. Phylogenetic analysis of the protease region of both specimens with subtype F clustered with Puerto Rican F rather than with Brazilian or Romanian F [7].

The patient infected with subtype A (BX381) was an African-American man born in the United States who had lived in the Bronx since the 1950s. His first HIV-positive test was in May 1992, with a negative HIV test result in 1991. He estimated that he had sex without a condom with ~300 women between 1948 and 1992 but denied sex with known foreign nationals or travelers to places outside the United States, injection drug use, and receipt of a blood transfusion. He did not report ever traveling outside the United States.

The person infected with subtype F (BX434) was an African-American woman born in New York City who had lived in the Bronx since the 1960s. Her first HIV-positive test was in January 1997. She had no prior HIV-negative test. She reported having sex without a condom with 5 different men between 1978 and 1997 but denied sex with known foreign nationals or travelers to places outside the United States, injection drug use, and receipt of a blood transfusion. She did not report ever traveling outside the United States.

The person infected with recombinant subtype B/F (BX823) was an African-American man born in the United States who had lived in the Bronx since the 1960s. His first HIV-positive test was in 1988: he had a negative HIV test result in 1987. He reported sex without a condom with 10 different women and 10 different men between 1980 and 1988. These included 1 man from Puerto Rico with whom he had repeated sexual contacts from 1984 through 1987. No other sex partners were known to be foreign nationals or to have traveled outside the United States. He reported no injection drug use or blood transfusion and no travel outside the United States.

Discussion

This report is among the first to identify infections with HIV-1, group M, non-B subtypes in US residents with no history of travel outside the United States. It includes the first report of a recombinant HIV-1 isolate on the North American continent. Infection with non-B subtypes was detected in 2 of 119 persons without contact with foreign nationals or foreign travelers and in 1 of 137 persons with such contact. In addition, none of these 3 persons had contacts that would put them at risk of acquiring HIV while traveling outside the United States. Thus, the evidence supports the transmission of non-B subtypes in the United States.

To maximize the opportunity to detect non-B subtypes, we intentionally included a large sample of persons with a history of sexual or needle-sharing contact with persons from other countries. Thus, the prevalence of non-B subtypes we detected should not be considered representative of the South Bronx or the US population. We attempted to include persons who were born in West Africa, but none were eligible because those we approached had moved to the United States after age 15. All
of the persons in our study who were born outside the United States were from Caribbean islands.

The serology screen we used is a sensitive method for detecting subtype B HIV-1 in the V3 loop [17]. However, the specificity of the V3 loop serology alone is not sufficient to exclude recombinant viruses. We cannot exclude the possibility that some of the 195 specimens that were classified as subtype B by serology, and thus did not undergo sequencing of gp41 and protease, may have been recombinant viruses. Thus, the detection of 3 non-B subtypes from our sample is a minimum estimate.

Artenstein et al. [6] reported the first well-documented instance of transmission of a non-B HIV-1 subtype in the United States. That case involved a US serovicea who had been infected with a subtype E virus while deployed in Thailand in 1993 [3] and who transmitted the virus to his wife in 1995. In an earlier study at BLHC, subtype A infections were confirmed for 2 persons in the South Bronx: a woman who had not traveled outside the United States but who reported having had sex partners who had spent time in the Caribbean, Latin America, or Africa and an African man who may have acquired his infection in Africa [7]. Brodine et al. [5] reported 1 US serovicea who apparently acquired a subtype E infection from a US serovicea while in the United States.

Other reports of HIV-1, group M, non-B subtypes in the United States have been published, but these infections were in persons who were most likely infected abroad [2–5]. The first reported case was a subtype D infection in a student from Zaire (now the Republic of Congo) who had moved to Alabama in 1988 [2]. Another case, detected in Maryland, was a subtype G infection in a woman of West African origin who was likely exposed to HIV in Africa [4]. Brodine et al. [3] published a case series of 5 US seroviceas who contracted HIV-1 infection while overseas: 3 subtype E infections were acquired in Thailand between 1989 and 1993, 1 subtype D infection was acquired in Kenya in 1987, and 1 subtype A infection was acquired in Uganda in 1992 or 1994. A later report documented 6 additional subtype E infections acquired in Thailand among US seroviceas [5]. HIV-1, group O infection has also been detected in persons in the United States who probably acquired the infection before their arrival [4, 18]. Active national surveillance for type O infections has shown that type O infections are rare in US residents [9].

Reports are increasing of the introduction of a wide variety of HIV variants into many countries [8, 19–33]. Such growth in variants seems to be the inevitable result of immigration and travel for military, business, or personal purposes [21, 25, 26]. The spread of diverse HIV-1 strains has also been documented within European countries [21, 25].

The growing diversity of HIV-1 strains in the United States may have public health implications for HIV diagnostic testing, antiretroviral therapy, vaccine development, and ensuring blood safety [34–36]. Most HIV-1 tests used to diagnose infection or to screen blood in the United States are based primarily on subtype B (Western). Although there is no evidence that the technical performance of HIV diagnostic tests now marketed in the United States differs by subtype (except for the slightly reduced sensitivity for group O [37]), development of new HIV diagnostic tests should consider technical performance in detecting non-B subtypes.

HIV-1 vaccines based on subtype B may not provide protection against non-B subtypes [35]. If surveillance confirms an increasing prevalence of non-B subtypes in US communities, the development of vaccines intended for use in the United States that provide protection against non-B subtypes may be warranted. Although in vitro data suggest similar responses to antiretroviral drugs among subtypes [36], clinical studies to assess these observations are warranted. In 1997, the CDC began a sentinel surveillance system for non-B HIV infections in persons with newly diagnosed HIV infections. Such surveillance will generate more population-based information on which to base public health decisions.

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References


