HIV Type 1 Superinfection with a Dual-Tropic Virus and Rapid Progression to AIDS: A Case Report

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Background. The occurrence of human immunodeficiency virus type 1 (HIV-1) superinfection has implications for vaccine development and our understanding of HIV pathogenesis and transmission.

Methods and Results. We describe a subject from the Multicenter AIDS Cohort Study who was superinfected with a dual-tropic (CXCR4/CCR5-utilizing) HIV-1 subtype B strain between 0.8 and 1.3 years after seroconversion who had rapid progression to AIDS; the subject developed Pneumocystis pneumonia 3.4 years after seroconversion, as well as multiple other opportunistic infections. The superinfecting strain rapidly became the predominant population virus, suggesting that the initial and superinfecting viruses in this individual differed in virulence. However, we found no molecular epidemiological evidence in the HIV database to suggest that this strain had been found in other individuals. In addition, this subject’s HIV-1 viral load and pattern of human leukocyte antigen and coreceptor polymorphisms only partially explained his rapid disease progression.

Conclusions. Additional studies are needed to determine whether superinfection itself and/or infection with a dual-tropic virus causes rapid disease progression, or whether certain individuals who are innately more susceptible to rapid disease progression also lack the ability to resist the challenge of a second infection. This case appears to support the latter hypothesis.

The occurrence of superinfection with a second strain of HIV-1 has important implications for understanding both HIV transmission and the immunity elicited during HIV infection, as well as for the immunity required by an effective AIDS vaccine [1]. Superinfection occurs when a second viral strain infects an individual in whom primary HIV infection and seroconversion have already occurred and is distinguished from coinfection, in which both strains are present during primary infection. Superinfection and coinfection (collectively referred to as dual infection), have produced the numerous recombinant viral strains (referred to as circulating recombinant forms) that have increasing global epidemiologic significance [2, 3]. However, the frequency and pathogenic consequences of superinfection are largely unknown [1, 4–6]. Several reports convincingly document HIV-1 superinfection [7–22]; however, cases of superinfection may be rare, at least in certain populations [23, 24], and careful laboratory techniques are necessary to confirm them [25, 26].

There have been reports of HIV-1 intrasubtype [9–13, 17], intersubtype [7, 8, 14, 16, 18–21], and intergroup superinfection [15]. In addition, replacement of drug-resistant HIV by superinfecting wild-type virus [10], superinfection with drug-resistant strains [17, 22, 27], and superinfection leading to recombination between subtypes [18, 22, 28] have been described. Although several of the aforementioned studies observed an increase in HIV-1 plasma RNA load and a concomitant increase in the loss of CD4+ T cells at the time of superinfection, it remains unclear whether superinfec-
tion causes more-rapid disease progression, and if so, what the underlying mechanisms might be.

Primary infection with HIV-1 almost always occurs with strains that use the CCR5 coreceptor (R5 strains) [29–32]. Initial infection with CXCR4 coreceptor-utilizing (X4) HIV-1 strains (or dual R5/X4-tropic strains) is rare, but it can occur in individuals who are homozygous for a 32–base pair deletion in CCR5 [33, 34] and has been associated with more-rapid disease progression [35, 36]. A recent report and follow-up investigation of the so-called “New York case,” which occurred in an individual infected with a multidrug-resistant dual-tropic HIV-1 strain who experienced rapid progression to AIDS [37, 38], has rekindled interest in the causes of rapid HIV disease progression, such as host susceptibility, unusual strain virulence, and/or other factors [11, 39–45, 46].

In a recent study of dual HIV-1 infection, we found an association between infection with 2 HIV-1 strains and rapid disease progression [11]. In that study, 1 subject had evidence of superinfection with a second strain of HIV-1 subtype B. In the present study, we further investigated the host-virus interactions in that subject and found that he was initially infected with a CCR5-tropic strain of HIV-1 and then was superinfected with a strain that was dual-tropic.

METHODS

Study subject. This case of superinfection was initially identified in the Multicenter AIDS Cohort Study (MACS) [47], as previously described [11]. Informed consent was obtained, and the study protocol was approved by the University of Washington Human Subjects Committee in accordance with the human experimentation guidelines of the US Department of Health and Human Services.

Subject samples, virus isolation, heteroduplex mobility analysis, PCR, cloning, and sequencing. Cryopreserved samples of plasma and/or PBMCs were obtained at 0.3, 0.8, 1.3, and 3.3 years after seroconversion and were screened for superinfection using a combination of heteroduplex mobility analysis and virus sequencing, as described elsewhere [11, 48, 49]. HIV-1 plasma viral RNA and PBMC viral DNA were isolated as described elsewhere [49, 50]. Nested PCR of the HIV-1 C2-V5 envelope (env) region was performed using end point dilution to avoid template resampling bias [51], as described previously [49, 50]. PCR products were cloned and sequenced using standard methodologies.

Phylogenetic analysis. All sequences were assessed for potential sample mix-up and contamination by established techniques, as described previously [52]. Each sequence was aligned with reference sequences from the HIV Sequence Database [53] with ClustalW [54], followed by manual adjustment using MacClade software, version 4 [55]. Pairwise evolutionary nucleotide distances were estimated using distance-based methods. Neighbor-joining and maximum likelihood methods were used to estimate phylogenetic trees using PAUP software, version 4.0 (Sinauer) [56]. Phylogenetic estimates of the most recent common ancestor of the superinfecting strain were made using maximum likelihood criteria, as previously described [57–59]. The deduced amino acid sequence of the V3 loop of the most recent common ancestor from the superinfecting strain was used to assess coreceptor use (see below). The criteria used to define dual HIV-1 infection were previously described [11].

Detection of HIV-1 superinfection. In brief, HIV-1 plasma viral RNA obtained 0.3, 0.8, 1.3, and 3.3 years after seroconversion and viral DNA from PBMCs obtained 0.3, 1.3, and 3.3 years after seroconversion were available for analysis from this subject. Heteroduplex mobility analysis [48] of the HIV-1 C2-V5 env region revealed a unique length polymorphism (615 base pairs vs. 597 base pairs) that distinguished the 0.3-year and 0.8-year virus samples from those obtained at 1.3 and 3.3 years after seroconversion (data not shown). There was no evidence of the later viral variant in the 0.3- and 0.8-year samples by heteroduplex mobility analysis at a limit of detection for low-frequency variants of 1%–2% [48, 60]. Phylogenetic analysis of 131 HIV-1 C2-V5 env region sequences from plasma and PBMC also suggested superinfection, in that there was no evidence of the superinfecting strain before 1.3 years after seroconversion (limit of detection of <1 [2.6%] of 39 clones from the 0.3- and 0.8-year samples). In addition, initial and superinfecting strain-specific PCR was used to further confirm superinfection. PCR primers were designed that were highly specific for either the initial or superinfecting strain. There was no cross-amplification of strains by these primers to a level of detection of between 1 and 10⁴ copies of input template. There was no evidence of the superinfecting strain in the 0.3-year (plasma and PBMC) and 0.8-year (plasma) samples with a limit of detection of 1–10 copies of superinfecting strain per PCR reaction of PBMC DNA or plasma. A total of ~2.85 µg of PBMC DNA (equivalent to ~438,900 cells) and 0.1 mL of plasma were screened for evidence of the superinfecting strain in the 0.3-year and 0.8-year samples using strain specific PCR. The possibility of subject-sample mix-up was excluded by molecular HLA class II typing of the pre- and post-superinfection samples [61].

CD4+ T cell trajectory determinations. Acquisition of the second virus could have caused an acceleration of the decrease in CD4+ T cells. We therefore determined whether the subject’s CD4+ T cell counts and CD4 cell percentage exhibited a significant change (inflection) in rate of decrease over time by performing a quadratic least-squares fit of the CD4+ T cell count and CD4 cell percentage on time and determining whether the coefficient of the quadratic term was significantly different from zero. We performed similar analyses using CD3+ T cell counts.
and CD3+ cell percentage. The most likely time of inflection was determined by the method of Gange et al. [62], using a routine written in R [63].

Coreceptor use assays and envelope V3 loop genotypic prediction of R5/X4 and non-synctium-inducing (NSI)/synctium-inducing (SI) phenotype. Phenotypic HIV coreceptor use was determined using the Trofile HIV Co-Receptor Tropism Assay (Monogram Biosciences) on bulk plasma isolates recovered 0.3, 0.8, 1.3, 1.8, 2.4, and 2.8 years after seroconversion. Genotypic analysis of the V3 loop of env was performed on independent clones from 0.3, 0.8, 1.3, and 3.3 years after seroconversion, as previously described [64, 65]. In brief, V3 loop sequences were predicted to be X4/SI if they had a basic amino acid residue (K or R) at position 11 or 25 (referred to as a canonical mutation) [66] or had a position-specific scoring matrix score consistent with a predicted X4 or SI phenotype [64, 65].

Molecular HLA typing and HIV coreceptor polymorphism analysis. Host genetic polymorphisms (in HLA and HIV coreceptor genes) reported to alter disease progression rates were determined, as described elsewhere [67–69].

Nucleotide sequence data. HIV-1 sequences generated from this study were submitted to GenBank (accession numbers, EF579969–EF580099).

RESULTS

The subject was a 39-year-old Hispanic man who was initially infected in 1992. His disease course and treatment history are summarized in figure 1 [11]. The time from seroconversion to a CD4+ T cell count <200 cells/µL was 2.4 years, and his first clinical AIDS-defining event (Pneumocystis pneumonia) occurred 3.4 years after seroconversion. Superinfection occurred between 0.8 and 1.3 years after seroconversion. In the 6-month period preceding his infection with the second strain (at the visit 1.3 years after seroconversion), the subject reported sexual activity with 27 different male partners, including unprotected oral and anal receptive and insertive sex. He did not report any symptoms consistent with an acute retroviral syndrome in that period. At the 2 visits before and after superinfection, the patient’s CD4+ T cell counts were 558 cells/µL and 360 cells/µL, respectively. Regression analysis of the CD4+ T cell count trajectory suggested that his rapid decrease in CD4+ T cells (in both absolute cell count and CD4 cell percentage) began with seroconversion ( ) (i.e., predated the superinfection) and that there was no detectable CD4+ T cell inflection at the time of superinfection (figure 1). Similar results were found for CD3+ T cell trajectories.

By phylogenetic analysis, both the initial and superinfecting strains were subtype B (figure 2) [11]. Strain-specific PCR de-
tected no superinfecting strain in plasma or PBMCs before 1.3 years after seroconversion (figure 3). The superinfecting strain rapidly overtook the initial virus in plasma between 0.8 and 1.3 years after seroconversion (figures 1–3). In PBMCs, the superinfecting strain predominated at 1.3 years after seroconversion (1978 copies per 10^6 PBMCs vs. 11 copies per 10^6 PBMCs), and there was no evidence of the initial strain at 3.3 years after seroconversion (figures 1 and 2). Between the last visit at which the patient was infected with only the original strain and the first visit at which the patient was infected with the superinfecting strain, the plasma HIV-1 load increased from 25,466 copies/mL to 87,176 copies/mL, and the PBMC HIV DNA concentration increased from 327 copies per 10^6 PBMCs to 1989 copies per 10^6 PBMCs.

The initial and superinfecting viruses used different HIV coreceptors, as determined by both functional phenotypic and genotypic analyses (see Methods; tables 1 and 2). The initial infecting virus consisted solely of R5 variants by both methods, whereas the superinfecting virus population was dual-tropic (R5/X4) by both methods (tables 1 and 2). The estimated most recent common ancestor of the superinfection strain was predicted to be X4-tropic by genotypic analyses (table 2).

There was no unusual genetic variation within either the initial or the superinfecting strains. The mean pairwise genetic distance between the initial and superinfecting strains in the C2-V5 region of the envelope gene was 18.6% (range, 16.6%–22.4%). The initial and superinfecting strain did not diverge from each other significantly more than expected for random sequences from the HIV Sequence Database (data not shown) [53]. The superinfecting virus was not epidemiologically linked to any sequence in the HIV Sequence Database (data not shown) [53]. Unfortunately, no formal partner investigation could be undertaken, given the retrospective nature of this study, the fact that the subject is deceased, and the lack of data regarding partners. By molecular typing for HLA and HIV chemokine coreceptor (CCR2 and CCR5) polymorphisms, the su-
Figure 3. Strain-specific PCR in plasma and PBMCs. Strain-specific PCR primers that specifically amplify either the superinfecting (SI) strain (upper panel) or the initial strain (lower panel) were used to amplify the C2-V5 envelope region of HIV-1 from plasma (obtained 0.3, 0.8, 1.3, and 3.3 years after seroconversion) and PBMCs (obtained 0.3, 1.3, and 3.3 years after seroconversion). PCR products were run on 1% agarose gels and stained with ethidium bromide. Each lane contains the equivalent of \( \sim 0.475 \) \( \mu \)g of PBMC DNA and \( \sim 17 \) \( \mu \)L of plasma. Representative gels are shown.

perinfected subject was found to carry HLA-A*0201/*0202, B*4901/*08, C*07/*07, DRB1*0101/*03, DRB3*0101, DQA1*01/*0501, DQB1*02/*0501 and CCR2-CCR5 genotype human haplogroup C/human haplogroup E. Partial homozygosity at class I loci and carriage of an human haplogroup E haplotype suggested that predisposing genetic factors may have contributed to a more rapid disease course irrespective of superinfection.

DISCUSSION

This study extends our initial report of the first known case of HIV-1 superinfection in a subject with rapid disease progression; we show here that he was superinfected with a dual-tropic virus [11]. In reports of HIV-1 superinfection to date, disease progression could not be directly assessed, because subjects either did not have long-term follow-up or were treated with HAART. In several reports of HIV-1 superinfection [7, 10, 13], there was a marked increase in plasma viral load and rapid decrease in CD4+ T cell count coincident with the emergence of the superinfecting strain. We detected a \( \sim 3.5\)-fold increase in viral load in our patient near the time of the emergence of the superinfecting strain, but no evident acceleration of the already high rate of decrease in CD4+ T cell count.

In this case, both “phenotypic” (Trofile HIV Co-Receptor Tropism Assay) and “genotypic” (V3 loop position-specific scoring matrix and 11/25 rule) methods demonstrated initial infection with an R5-tropic strain and superinfection with a dual-tropic population. However, neither assay can distinguish whether individual clones from the superinfecting strain were dual-tropic or a mixed X4 and R5 population. We cannot completely rule out the possibility that an R5 virus was transmitted during superinfection and quickly evolved into an X4/dual-tropic variant. The only definitive way to rule this possibility out would be to have a sample (which we do not) from the moment of SI transmission that shows only an X4 or dual-tropic population or a mixed population. However, we think that the possibility that an R5 virus was transmitted during superinfection and quickly evolved into X4/dual-tropic strain is extremely unlikely, for the following reasons: (1) the majority of the sequences at the year 1.3 visit (34 [72%] of 47) are predicted to be X4/dual-tropic; (2) using maximum-likelihood phylogenetic methods, the predicted most recent common ancestor of the superinfecting lineage for the 1.3-year visit is predicted to be X4/dual-tropic/SI (strongly arguing that the R5 tropic variants actually evolved from the X4/dual-tropic/SI variants and not the other way around); (3) at 3.3 years after seroconversion (2 years after superinfection), the majority of the sequences (22 [92%] of 24) were predicted to be R5, suggesting evolution from X4 to R5 from the time of superinfection; and (4) there were no predicted X4 viruses (using the 11/25 rule) in PBMCs at 1.3 years in the initial infecting strain by strain-specific PCR.

Viruses that use CXCR4 as a coreceptor (X4 viruses) or that form syncytia on MT2 indicator cells in vitro (SI viruses) have been associated with a rapid decrease in immune system function and faster disease progression [70, 71]. However, primary HIV infection generally occurs with R5/NSI viruses and has only rarely been reported with X4/SI or dual-tropic viruses, despite the fact that X4 viruses are found in at least 50% of individuals with HIV infection and are likely, therefore, to be present in many inocula resulting in transmission [29, 30]. Superinfection with a dual-tropic strain suggests that this individual may have been less able to restrict the population of X4/SI viruses in the superinfecting inoculum than he would have been if he had had an intact immune system at the time of primary infection. The subject may also have been more
intrinsically susceptible, not only to superinfection per se, but also to infection with X4/SI viruses. Interestingly, by 3.3 years after seroconversion (2 years after superinfection), when the patient’s CD4+ T cell count had decreased to 77 cells/μL, the predominant virus population appeared to have reverted to R5/NSI. These R5/NSI variants evolved from the superinfecting dual-tropic population and did not represent a recrudescence of the initial strain (figure 2). This phenomenon has been observed in other subjects with end-stage disease [49], as well as in patients treated with ART [72], and it may reflect selection pressure on the virus, induced by a severe decrease in the number of T cells displaying CXCR4 (specifically, naive T helper cells). In addition, the X4/SI phenotype may have contributed to the selective replacement of the initial strain. Biological studies of the 2 strains will be necessary to confirm differences in their replication ability and/or virulence.

This superinfected subject displayed some potential genetic susceptibility to rapid disease progression. His chemokine receptor genotype human haplogroup C/human haplogroup E has been associated with increased transmission and faster disease progression in white individuals [68] and, specifically, in people of Hispanic origin [73, 74], and homozygosity at HLA class I loci has also been associated with more-rapid disease progression [75]. Whether these genetic characteristics predisposed this individual to superinfection or infection with a dual-tropic virus awaits further studies of the risk factors for these events.

Reports of transmission of drug-resistant strains have increased recently [76–78], and superinfection can occur with drug-resistant strains [17, 22, 27]. In addition, the much-publicized case of rapid progression in a man infected with a dual-tropic, multidrug-resistant HIV-1 strain demonstrates that both of these harbinger of a poor prognosis may coexist in the same infecting virus population [37, 38]. However, in our case, the superinfecting HIV-1 strain was unlikely to harbor significant drug-resistant mutations, because our subject was antiretroviral naive at the time that he was superinfected (sometime between 1992 and 1993, in the pre-HAART era). Unfortunately, samples were unavailable to perform antiretroviral resistance genotyping or phenotyping, and we cannot rule out the presence of some nucleoside reverse-transcriptase inhibitor (e.g., zidovudine and didanosine) resistance mutations in the superinfecting strain.

We previously demonstrated a correlation between dual HIV-1 infection and very rapid disease progression [11]. However, it is not clear whether superinfection leads directly to rapid progression, or whether some hosts whose disease is progressing rapidly are also less able to prevent infection with a second strain. The present case argues for the latter possibility, for several reasons: (1) the subject had a rapid decrease in his CD4+ T cell count that began during primary infection and preceded superinfection, (2) X4 viruses are uncommon infecting strains, (3) we found no molecular epidemiological evidence in the HIV Sequence Database [53] to suggest that the superinfecting strain from this individual has been found in other individuals (unfortunately, no formal partner investigation could be undertaken, given the retrospective nature of this report, the fact that the subject is deceased, and the lack of data regarding partners), and (4) the individual carried genetic markers related to poor immune response and poor prognosis.

The implications of superinfection for disease progression, as well as for HIV-1 therapy, transmission, and vaccine development, remain to be clarified. However, as additional cases
of superinfection emerge, the perception that host immunity induced by primary HIV-1 infection is generally protective against acquisition of a second virus or that correlates of immunity inferred from infected subjects predict those desirable for a vaccine may need to be reevaluated [1, 5]. Finally, our findings argue for further investigation of the underlying causes of superinfection and rapid disease progression.

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