Transmission of Two Distinct HIV Type 1 Strains to an Individual That Were Harbored for Many Years by Another

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Abstract

The concept of transmission bottlenecks in HIV-1 infection is well established. Coinfections and superinfections have been increasingly documented and provide a founding cause for the expansion of viral diversity through recombination. It is still relatively unclear how HIV-1 will propagate and evolve in individuals infected with more than one viral strain. Here we report on the parallel transmission of genetically distant viral strains cocirculating in one individual over many years to a single recipient.

MUCOSAL TRANSMISSION PATHWAYS for HIV-1 are not well understood, providing a hurdle for the development of an effective vaccine or microbicide preventing infection. The physiology of the mucosal tissue and the cell types of the innate as well as adaptive arms of the immune response present will undoubtedly be involved in determining whether a virus undergoes transmission.^{1–3} It has been well documented that specific virus strains undergo transmission with CCR5 using viruses being exclusively transmitted.⁴ Even with donors infected with CCR5 using viruses a restricted quasi-species of viruses can be found in the recipient after infection at a time point before seroconversion and the induction of neutralizing antibodies.⁵ This bottleneck indicates that there are factors restricting transmission without excluding a stochastic character of HIV-1 transmission during the sexual encounter. However, to this day it remains unclear whether it is the virus, the host, or the host/virus that is the dominating factor in the transmission bottlenecks. Restrictions to transmission based on viral genotype/phenotype are encouraging for vaccine and microbicide development; however, it has been difficult to define common genetic restrictions among transmitted viruses.⁶

In this report we determine that diverse HIV-1 strains cocirculating in one individual for over 10 years can be identified within another positive individual. We initially identified an individual (Pat A) infected with two distinct HIV-1 strains, both subtype B viruses (solid symbols in Fig. 1A). This individual was a man having sex with men (MSM) with no steady partner and who first tested HIV-1 positive in December 1995.

He was found to have a CD4⁺ count of 440 cells/ μ l³ and a viral load of over 50,000 copies/ml (4.72 log₁₀ units) (solid symbols in Fig. 1B), which fell to below the detection limit after initiation of therapy (zidovudine/lamivudine/ritonavir) in December 1996. In 1998 the therapy regime was changed to stavudine/lamivudine/nelfinavir, although resistant virus did not arise. He was lost to follow-up in November 1999 and the plasma RNA load at his last visit was undetectable, with a CD4⁺ cell count of 460 cells/ μ l³. Subsequently he returned to the clinic 5 years later in the middle of 2004. This time he had an elevated viral load (5.97 log₁₀ units) and low CD4⁺ cell count (90 cells/ml³). For both his first visit in 1995 and his return visit in 2004, from stored plasma we amplified the gp120 region of the envelope corresponding to the 6600–7754 HXB2 nt location.

Following cloning of the reverse transcriptase polymerase chain reaction (RT-PCR) amplicon in the TOPO-TA cloning system from Invitrogen and DNA sequence analysis of at least 10 individual clones it was revealed that he was carrying two subtype B strains as shown by the phylogenetic analysis of the gp120 envelope gene (Fig. 1A). The nucleotide sequences were aligned in respect to the predicted amino acid sequence of the corresponding reference alignment extracted from the Los Alamos HIV database⁷ and phylogenetic analyses were conducted using MEGA software⁸ (version 3.0) and the distance matrix was generated by Kimura's two-parameter estimation.⁸

Around the time Pat A returned to the clinic in August 2004 a newly infected individual (Pat E) presented with fever,

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FIG. 1. (A) Phylogenetic analysis of the virus clonal sequences isolated from Pat A (solid symbols) and Pat E (open symbols). Neighbor-joining/Kimura two-parameter phylogenetic analysis was performed using the MEGA software package.⁸ The two cocirculating strains are indicated by the gray arrowed lines. The three different symbols indicate the different time points that were analyzed: A1-Oct/1996, A2-Dec/1998, A3-Oct/2004, E1-Sep/2004, E2-Dec/2004, and E3-Sep/2005. Sequences taken from the Los Alamos database were used as references. Numbers by the branches represent bootstrap values out of 100 replications. (B) Plasma viral RNA load measurements of Pat A (solid symbols thin line) and Pat E (open symbols thick line). The larger circles over the symbols indicate the time points investigated.

diarrhea, skin rash, and pain in his joints. Pat E, who claimed to be heterosexual with no steady partner, harbored HIV-1, which could be linked to virus from Pat A by RT-Prot sequence analysis performed as a routine baseline antiretroviral treatment (ART) resistance test. The viral load follow-up testing of this individual demonstrated a classic seroconversion profile with a high peak of viremia ($6.7 \log_{10}$ units) that remained high at around $5 \log_{10}$ units after set point (thick line Fig. 1B). We selected three plasma samples for the analysis of

the gp120 envelope as performed for Pat A: one from his first visit (during peak viremia), another at 12 weeks following the set point, and the other 38 weeks later. The gp120 sequence analysis revealed the presence of the same two virus strains in Pat E as observed in Pat A.

The genetic relatedness of the viruses found in Pat E was, as expected, greater for virus isolated from Pat A when he returned to the clinic in 2004 (less than a 1% difference). This was within a 2-month period of Pat E being diagnosed with

acute HIV-1 infection. Phylogenetic analysis of the sequences analyzed did not detect both strains during peak viremia in Pat E. In the first available sample (E1) we found strain II only, but found both strains in plasma analyzed at the beginning of the set point period 12 weeks (E2) after first being seen in the clinic when presenting with symptoms of acute infection as well as within plasma taken at 38 weeks (E3). However, with standard RT-PCR and nucleotide primers designed to recognize only strain I we detected strain I not only in time points E2 and E3 but also in E1 (Fig. 1A), indicating that these two strains were present in subject E from the beginning of the infection. The fact that the sequencing approach did not detect both viruses at peak viremia may indicate small differences in fitness between the two viruses, although a limited number of cloned virus sequences was studied and we did not perform fitness comparisons.

It has been shown that both coinfection and superinfection by distinct strains occur^{9,10} and two viruses are found to be transmitted within a group of promiscuous individuals.¹¹ In this report we do not provide a conclusive epidemiological link between Pat A and E but we do provide evidence that both patients are infected with the same two distinguishable strains of HIV-1. Phylogenetic evidence shows that Pat A was carrying both viruses for over 10 years before they were transmitted to Pat E. In addition, the strains found in Pat E were genetically close to those circulating in Pat A around the time he was first infected. This finding leaves us with the assumption that Pat A is the source and that these two strains coexisted and survived in this individual while retaining similar abilities to transmit. This suggests that while host factors that restrict transmission cannot be excluded, the transmission factors can be defined by the virus as well. Amid the vast diversity of HIV-1, if we could associate viral genetic characteristics with transmission bottlenecks, we would be one step ahead in the future design of relevant immunogens.

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Author Disclosure Statement

No competing financial interests exist.

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