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HIV-1 superinfection with a triple class drug resistant strain in a patient successfully controlled with antiretroviral treatment

Running head: HIV-1 superinfection despite successful cART

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Abstract

We report a case of HIV-1 superinfection (HSI) with a clade B, triple class-resistant virus in a patient successfully controlling viremia with continuous cART started 8 years earlier during primary HIV infection. The course of HIV infection prior to HSI was monitored in both the source partner and recipient (8 and 11 years, respectively) and 4 years following HSI. This case report demonstrates re-infection with HIV-1 despite effective cART.

Background

HIV-1 superinfection (HSI), that is, infection with a second strain after the first has been established, has been reported since 2002 mainly involving HIV group M clades {1-6} and in a smaller proportion as inter-group recombinant forms {7-8}. Overall, HSI has been most often observed in untreated patients, during treatment interruption, and in seroconcordant couples with poor viral suppression {9}. Here we report the onset and 4 year follow-up of HSI with a triple class resistant clade B virus in a man on effective combined antiretroviral treatment (cART).

HIV superinfection

Briefly, blood samples were obtained from 2 men (M1 and M2) chronically infected with HIV-1 and sexual partners since 2006. M2 was diagnosed with HIV infection in 1994 at stage A2 with full Western blot seroconversion, and developed triple class antiretroviral drug resistance as a consequence of weak adherence to ART, leading to virologic failure. M2 experienced uncontrolled viremia (range: 3 to 4 logs of viral RNA) until September 2008 when a salvage treatment regimen reduced his viral load to undetectable for the first time (Figure 1a). M1 was diagnosed in 2000 with primary HIV infection, initiated cART in 2000, and remained on cART with undetectable viremia and no drug resistance mutations through the end of 2007 (Figure 1b). cART first regimen consisted of ZDV+3TC+EFV. In 2001 EFV was replaced by LPV/r due to EFV neurologic intolerance. Therapy was simplified in 2002

with the association of ZDV/3TC/ABC (Trizivir®). M1 was also a vaccine recipient in the therapeutic HIV vaccine trial TheraVac01 {10}. Briefly, the trial was an open-label one arm study that took place in Lausanne, Switzerland. All subjects (n=10) were immunized with NYVAC-B (Gag/Pol/Nef polygene of HIV IIIB, and Env clade B of HIV BX08) intramuscularly (10E7.4 CCID50/mL) at weeks W0 and W4. M1 was assigned the code TH#04 and was immunized on May 17 (W0) and on June 14 (W4) 2006, respectively.

In February 2008, M1 presented with a plasma viral load of 280 copies/mL that increased over the following year. Genotypic analysis from 2008 onward revealed 25 new drug resistance mutations to NRTI (6), NNRTI (5) and PI (14). Of note, this resistance profile shared 22 of 23 mutations (96%) found contemporaneously in M2. The shared mutations included NRTI resistance mutations 41L, 74I, **184V**, 215Y, NNRTI mutations, 98G, **103N**, 108I and PI mutations 10V, 13V, 20R, 32I, 33I, 46I, 47V, 50V, 71I, 77I. M1 also developed 75T (NRTI), 115/Y (NNRTI) and 82A (PI) polymorphisms.

Molecular and Phylogenetic analyses

We obtained viral gene sequences from M1 from July 2000 (N=11 ~9kb near full-length genomes, NFLG), February 2008 (N=12 gag and env genes) and May of 2008 (N=14 gag and N=16 env genes), and from M2 in January (N=10 NFLG) and March of 2008 (N=7 NFLG). All 86 viral sequences were assigned to clade B. Additionally, all sequences from M1 obtained from 2008 clustered with M2 sequences from the same year and were phylogentically unrelated to M1 sequences from 2000 (data not shown). This indicated HSI of M1 with substantial or complete replacement with virus from M2. No recombination between the M1 and M2 strains was observed.

Four years follow-up

M1's viral load increased from undetectable to >3 logs after HSI, decreasing progressively from 2009 to 2012 without cART modification. A continuous drop of CD4+ cells of at least

10% was observed from time of HSI detection in February 2008 (30.8%) through April 2011 (18%). However, in April 2012, M1's CD4+ count recovered to 25.4% (757 cell/mm^3) (Figure 1b). Patient M2's follow-up was taken over by a general practitioner in 2009 after 3 consecutive undetectable viral loads following start of salvage antiretroviral treatment (Figure 1a).

Discussion and conclusions

We detected superinfection and replacement by HIV-1 clade B triple class-resistant virus in a patient on long-term cART controlled infection, with the initial indicator of HSI being a detectable and increasing viral load.

Other resistance mutations not found in the superinfecting strain also emerged following HSI, though their origin is unclear. They could have been present as minority populations prior to superinfection or transmitted with the superinfecting strain but below the detection level of our assessment of M2's quasispecies (no additional specimen was available for massively parallel sequencing). Despite HSI with a triple class resistant virus, M1's antiretroviral treatment remained unchanged. During 4 years of additional follow-up, a continuous drop of viral load occurred, followed by CD4+ recovery in the last year (Figure 1b). The impact of HSI in CD4+ decline and disease progression following subtype B coinfection was initially suggested by Gottlieb and colleagues {11}. Evidence of CD4+ T-cell decline as the initial indicator of HSI was also reported in untreated patients during primary HIV infection as well as in untreated elite controllers {12-14}.

In contrast to previous reports of superinfecton among long-term known seroconcordant couples undergoing ART {9}, this report underscores the fragile chemoprophylactic barrier exerted by ART despite excellent adherence. Apart from the complex resistance profile of the superinfecting strain present in patient M2, we cannot rule out the role of viral escape to

preexisting CTL or antibody responses in the establishment of the second infection, which involves the same clade and therefore, to some extent challenged similar immune signatures in the superinfected individual, as reported elsewhere {15-16}. Of interest, M1's HIV-specific T-cell responses were enriched following NYVAC-B immunizations (weeks 0 and 4) and 21 months prior to superinfection {10}. Briefly, two novel Env/Pol vaccine-induced responses emerged at W2 and remained present throughout the study with a clear decline by W48 {10}, greater than 1 year prior to HSI. The protective role of these vaccine-induced responses to prevent a second infection with a resistant virus, as well as their longevity, remains unproven. Haplotypes such as HLA-B3503 have been shown to be associated with HIV-1 superinfection susceptibility as a consequence of late or weak immune response priming {15}. In this regard, patient M1 carries HLA haplotype A*30/23, B*35/44, DRB1*14/04 (performed by PCR-SSO using LabType® kit on the Luminex System). He also had no CCR5-delta 32 mutations (in-house PCR modified from Wilkinson DA et al., 1998) {17}.

In summary, patient M1's superinfection onset agrees with current knowledge on host factors and re-exposure to a resistant strain, and demonstrates the previously unexpected scenario of re-infection during well-established chronic infection, despite continuously suppressed viremia with cART started during primary HIV infection.

Acknowledgments

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Sequence Data

Nucleotide sequences were deposited in GenBank and are available under Accession numbers KC797171 - KC797229.

Footnotes

Author contributions: E.C, G.P, J.I.M, M.C and P.A.B designed research; E.C and H.Z performed research; E.C, H.Z, J.I.M, M.C and P.A.B analyzed data; and E.C, G.P, J.I.M, M.C and P.A.B wrote the paper.

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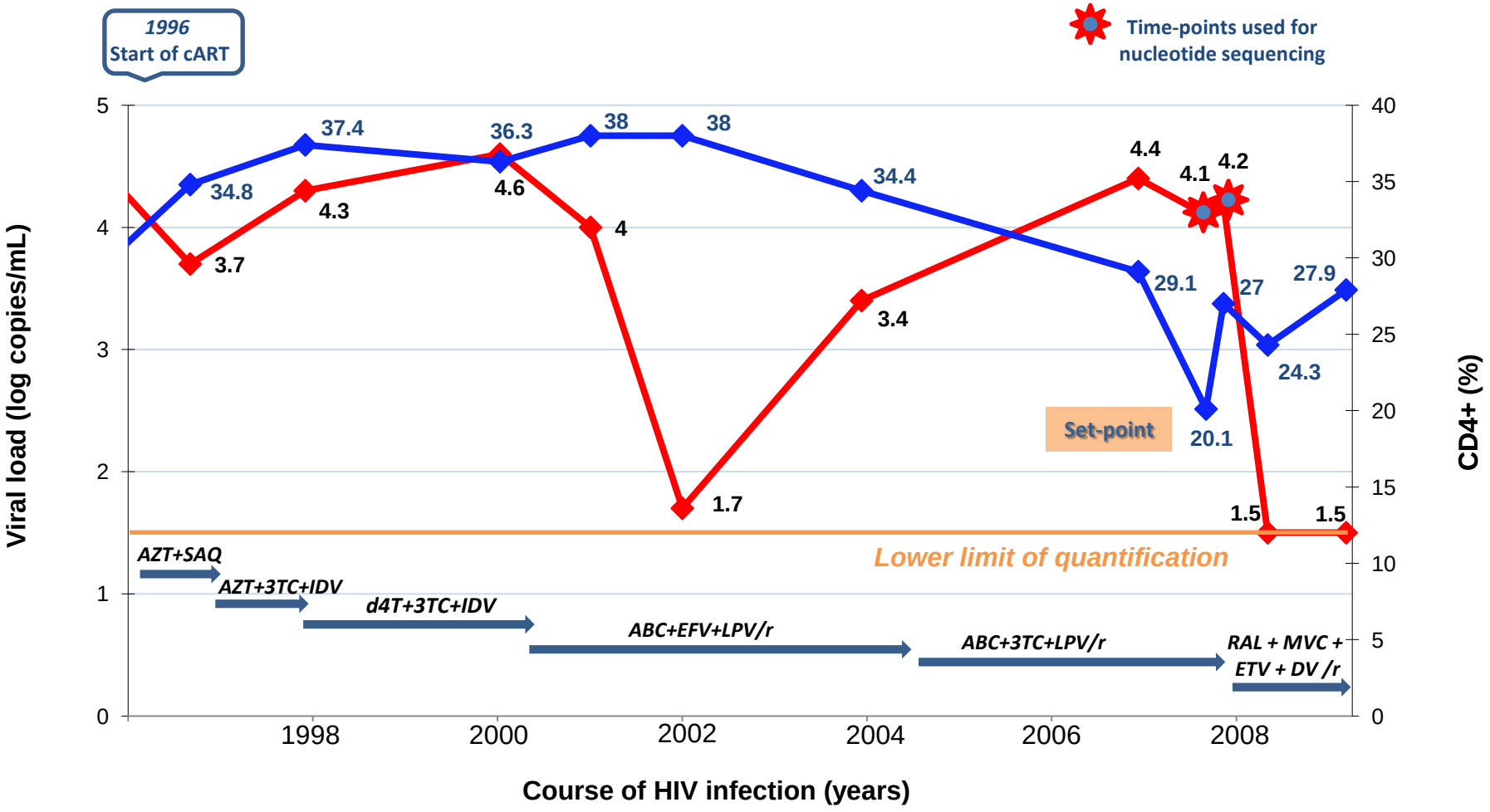
1a. Patient M2: Source of superinfection



1b. Patient M1: Recipient of superinfection