HIV-1 Superinfection in an HIV-2–Infected Woman with Subsequent Control of HIV-1 Plasma Viremia

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A human immunodeficiency virus type 2 (HIV-2)–infected woman experienced asymptomatic superinfection with HIV-1 subtype AG. She did not have cross-neutralizing autologous HIV-1 antibodies before and shortly after HIV-1 superinfection. This evidence supports a mechanism other than cross-neutralizing antibodies for the mild course of HIV-1 infection in this woman.

Interclade and intraclade superinfection with human immunodeficiency virus type 1 (HIV-1) strains has been well documented during recent years. Incidence rates are estimated to reach 3%–8% per year (with higher levels during primary infection than during chronic infection), rendering superinfection a major challenge for HIV drug and vaccine design [1–11]. In contrast, HIV-1 and HIV-2 coinfection is observed relatively infrequently in regions where both strains cocirculate at high levels [11–13]. A prospective epidemiological study from Senegal suggested that HIV-2–infected individuals may be protected partially from HIV-1 infection [14, 15], although other studies in West Africa could not confirm these findings [16, 17]. In addition, a variety of in vitro studies have proposed potential protective mechanisms [14, 18–20]. A caveat of previous studies remains: HIV-1 and HIV-2 dual infections could rarely be attributed clearly to either coinfection or superinfection, because sequential sampling to prove superinfection was missing in most cases. Here, we describe an HIV-2–infected woman who was unambiguously superinfected with HIV-1. Informed consent to conduct this study was obtained.

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Case report. In December 2001, a 39-year-old pregnant woman from Benin presented to our hospital with preterm contractions. The results of an HIV-1/2/O screening test were reactive. Western blot analysis revealed a positive result for HIV-2 but a negative result for HIV-1. Plasma HIV-1 RNA was undetectable at that time (Roche Monitor HIV-1 assay, version 1.5; detection limit, 50 copies/mL). HIV-2 infection had already been diagnosed in December 1999 (figure 1). The infant was born by cesarean delivery in the absence of antiretroviral treatment in December 2001 and remained free of HIV infection. To monitor for HIV-2 viremia, a product-enhanced reverse transcriptase assay [21] was performed in January 2002. Reverse transcriptase activity of 1266 nU/mL was detected (HIV RNA level, ~24,000 copies/mL) [21] and was thought to be attributable to HIV-2, because the plasma HIV-1 RNA test results were previously negative. The baseline CD4 cell count at that time was 729 cells/μL (CD4 percentage, 48%). In May 2002, the CD4 cell count decreased suddenly to 340 cells/μL (CD4 percentage, 26%). Subsequently, the CD4 cell count increased to 578 cells/μL (CD4 percentage, 27%) and remained stable (440–600 cells/μL) for the next 5 years. In November 2005, a plasma HIV-1 RNA test was performed, the results of which demonstrated measurable viral RNA. The assay was repeated, and the results confirmed the reactivity. This result raised the questions of whether this was a rare case of HIV-2 infection that was detectable by the Roche assay [22], whether the initial serological diagnosis of HIV-2 infection had been incorrect, or whether the patient had HIV-1 and HIV-2 coinfection.

Sequencing of the amplicon and phylogenetic analysis revealed an HIV-1 sequence related to clade A. Thus, a retrospective analysis of stored samples was started. Western blot analysis from May 2002 revealed that, in addition to the previous HIV-2 infection, HIV-1 infection had been newly acquired (figure 1); in addition, plasma HIV-1 RNA was detectable at the low level of 540 copies/mL. A plasma sample from January 2002, a time point at which the HIV-1 Western blot result was still negative, was also tested for HIV-1 RNA, and a viral load of 722 copies/mL was detected. Thus, asymptomatic primary HIV-1 infection occurred in January 2002. Subsequently, the HIV-1 RNA level increased to 1760 copies/mL in October 2002, spontaneously decreased to 63 copies/mL in January 2005, and then oscillated between 60 and 660 copies/mL during the entire follow-up period (until the end of July 2008). No HIV-associated disease developed during the entire observation period of 6 years, and it was not necessary to start antiretroviral treatment. HIV-1 proviral DNA amplifications for

gag and *pol* genes were positive. Phylogenetic analysis of the entire *gag* p24 sequence revealed recombinant virus CRF02_AG. The results of several plasma HIV-2 RNA tests remained negative, whereas HIV-2 proviral DNA was undetectable by standard polymerase chain reaction (PCR) but was detected unambiguously by a high DNA input nested DNA PCR [23]. Phylogenetic analysis of the sequenced amplicon revealed HIV-2 subtype B.

A replication-competent isolate of the superinfecting strain was isolated after 1580 days of follow-up. Of note, no HIV-1–neutralizing activity was detectable in the patient's plasma samples before HIV-1 superinfection (figure 1). Thus, HIV-2 lacked the ability to induce a protective, cross-neutralizing activity against HIV-1. A significant anti–HIV-1–neutralizing activity evolved after several months of HIV-1 infection, consistent with previous reports [24, 25].

Assays used. For HIV-1 and HIV-2 antibodies, the following assays were used: HIV-1/2/O enzyme-linked immunosorbent assay (for screening), Western blot analysis (Genelabs Diagnostics), and line immunoassay (INNO-LIA HIV I/II Score; Innogenetics). For HIV-1 RNA, the Cobas Amplicor HIV-1 Monitor assay, version 1.5, was used. Retrospective analyses of HIV-1 RNA in plasma samples or proviral DNA in peripheral blood mononuclear cells (PBMCs) used in-house real-time PCR with primers in *gag* and *pol*. For HIV-2 RNA or DNA PCR, PCR with primers in the *gag* leader sequence was used [26]. To measure reverse transcriptase activity, an ultrasensitive particle-enhanced reverse transcriptase assay was used [21].

The superinfecting autologous HIV-1 isolate was isolated from CD4 cells obtained after 1580 days of follow-up, and the autologous plasma neutralization activity was measured in a PBMC-based neutralization assay as described elsewhere [27] .

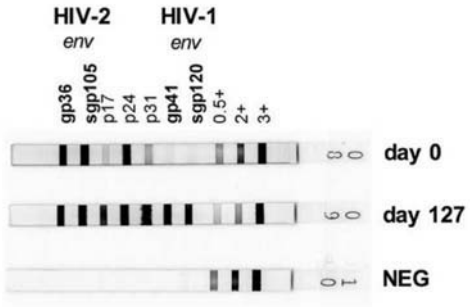

Figure 2. Line immunoassay with specimens obtained before and after human immunodeficiency virus type 1 (HIV-1) seroconversion. All specimens were tested retrospectively in parallel with strips of the same lot. Positions of HIV-1– or HIV-2–specific envelope antibodies are shown in bold. The specimen obtained on day 0 (in January 2002) showed seropositivity only for HIV-2. Seroconversion to HIV-1 is evident by the induction of HIV-1–specific envelope antibodies and by an increase of the reactivity to matrix protein p17 and endonuclease p31 in the specimen from day 127. NEG, seronegative reaction control.

Discussion. We report an unambiguous case of superinfection with HIV-1 CRF02_AG in an HIV-2–infected woman. Although the initial HIV-1 load was low (722 copies/mL) and peaked at only 1760 copies/mL, the sudden decrease in the CD4 cell count in this patient is consistent with primary HIV-1 infection and suggests that the superinfection occurred from December 2001 through January 2002, when results of the HIV-1 RNA assay were first positive but the results of INNO-LIA were negative for HIV-1–specific envelope antibodies (figure 2). HIV-1 and HIV-2 coinfection could clearly be ruled out by

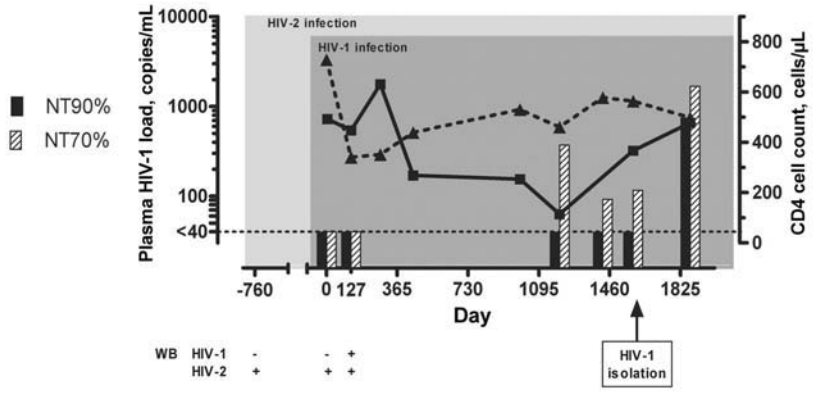

Figure 1. Synopsis of the time of events. Day 0 is defined as the day when diagnosis of human immunodeficiency virus type 1 (HIV-1) superinfection was made (in January 2002). The logarithmic scale delineates the longitudinal plasma HIV-1 load *(squares)* and neutralizing antibody titers of 70% (NT70%) and 90% (NT90%). The horizontal dotted line represents the cutoff value (<40 copies/mL) for nondetectability of any neutralizing activity against the autologous virus isolate. CD4 cell counts are represented by triangles. In addition, sequential Western blot (WB) results for HIV-2 and HIV-1, by the time of HIV-1 virus isolation from plasma samples, are shown to mark their overall temporal relationship.

identification of an HIV-2–positive Western blot result for this patient in December 1999. Of note, the patient did not experience any symptoms or signs of an acute retroviral syndrome. During the following 6 years, the HIV-1 load spontaneously decreased and oscillated at low levels (60–660 copies/mL), and the CD4 cell count fluctuated between 440 and 600 cells/μL (CD4 percentage, 23%–30%). It has to be considered that the infecting HIV-1 strain, as most isolates of CRF02_AG, contained a critical mismatch in 1 of the HIV-1 monitor primer binding sites [28]; thus, the viral load may have been underestimated. Despite repeated attempts, HIV-2 RNA remained undetectable in plasma, but persistence of HIV-2 infection could be documented by measuring HIV-2 proviral DNA levels in PBMCs with use of a supersensitive high-input procedure [23]. This case illustrates that, in an HIV-2–infected patient who encounters a sudden decrease in CD4 cell count, potential HIV-1 superinfection needs to be verified.

Previous studies have suggested that HIV-2 infection may partially protect patients from subsequent HIV-1 infection but not vice versa [15, 29]. Whether this is indeed the case is still under debate. Several clinical and basic research studies challenged these observations [16, 17], whereas other in vitro investigations located potential mechanisms by which HIV-2 infection could restrict or protect patients from HIV-1 infection, including CCR5 downregulation by HIV-2 [14], cross-reactive anti–HIV-1 antibody induced by HIV-2 [20], higher β-chemokine levels in HIV-2–infected patients [19], and viral interference at the transcriptional level [18]. To test the hypothesis that, in our patient, potentially cross-reactive anti–HIV-1 antibodies might have been induced by the previous HIV-2 infection, we longitudinally assessed neutralizing activity of the patient plasma against the autologous HIV-1 virus isolate. Of note, before and shortly after superinfection, no anti–HIV-1 plasma–neutralizing activity was detectable (figure 1). However, optimal antibody response could not be tested, because a viral isolate from the time of primary HIV-1 infection was not available.

In conclusion, our case is particular in several ways. First, it is a rare case of an unambiguously documented HIV-2 and HIV-1 superinfection. Second, the patient had asymptomatic acute HIV-1 infection with a low viral load, and subsequently, HIV-1 RNA levels remained low in the absence of any treatment for 6 years. HIV-1 superinfection only transiently led to a decrease in CD4 cell count, and for the entire follow-up period, no disease progression occurred; altogether, the course of the infection resembles chronic HIV-2 infection rather than HIV-1 infection. We could not detect cross-neutralizing antibodies against HIV-1 induced by HIV-2. Thus, mechanisms other than neutralizing antibody response were most likely responsible for this benign course of disease.

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Potential conflicts of interest. All authors: no conflicts.

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