Higher Frequency of Apparent HIV-1 Superinfection -- Sequentially Expressed Dual Infection (SEDI) -- in Recent Infection Compared to Chronic Infection

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Background

Sequential infection with variants of HIV-1, or superinfection, has been described in 5 published case reports involving persons who were recently infected or intermittently treated with antiretroviral therapy (Ramos 2002, Jost 2002, Altfeld 2002, Koelsch 2003, Smith 2004).

The frequency of appearance of highly divergent viruses in recent seroconverters has been estimated to be comparable to the overall incidence of new HIV-1 infections (Gonzales 2003, Tsui 2004).

In contrast, the detection of highly divergent viruses in chronically infected persons is rare.

Non-human primate research suggests that there is a window period of susceptibility to superinfection, lasting 4 weeks in pigtailed macaques (Otten 1999).

Mathematical models demonstrate that superinfection restricted to a short window period of susceptibility after initial infection could lead to highly prevalent recombinant infections due to secondary spread from dually infected persons (Gross 2004).

Objectives

To determine the frequency of HIV-1 sequentially expressed dual infections in a cohort of individuals who are identified as recently infected.

To evaluate partnerships to determine if sequential expression of dual infections is due to sequential infection (superinfection).

Methods

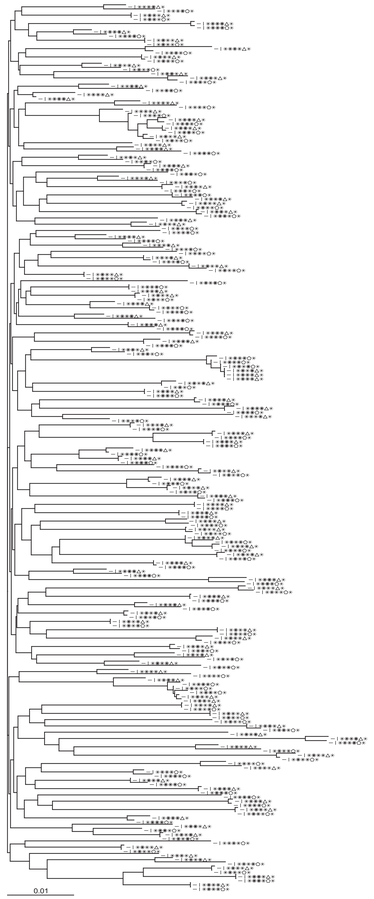
Acute and recent HIV-1 infection were diagnosed in the San Francisco Options Project using evolving serology, including seroconversion, evolving western blot, and less sensitive EIA testing. Population sequencing of the *pol* gene region was performed using a validated assay (TRUGENE, Bayer Healthcare). Phylogenetic analysis of baseline and follow-up timepoints was performed using bootstrapped neighbor-joining trees. To evaluate population dynamics, we used heteroduplex mobility assays (HMA) and inspected electropherograms to determine if the subsequent virus was evident in the baseline specimens. We have validated these methods at 1.5 to 3.0% sensitivity for minor variants.

Results

- 104 recently infected persons were analyzed at 2 or more timepoints, representing 195 person-years of observation.
- All viral sequences were subtype B.
- Highly divergent viral sequences appeared in a total of 4 cases over time, representing an incidence density of 2.1/100 person years (95% CI: 0.6% to 5.2%).
- Baseline and divergent sequence clusters were confirmed by independent analysis of specimens from additional timepoints.
- A divergent virus appeared between 120 and 168 weeks after baseline in one case.
- A 3-class drug-resistant HIV-1 variant was overgrown by a drug-susceptible viral variant in one case. Genotypic markers of nnRTI resistance (RT K103N or RTV108I) appeared in 2 cases.
- Viral load increased in all 4 apparent superinfection cases.

Figure 1

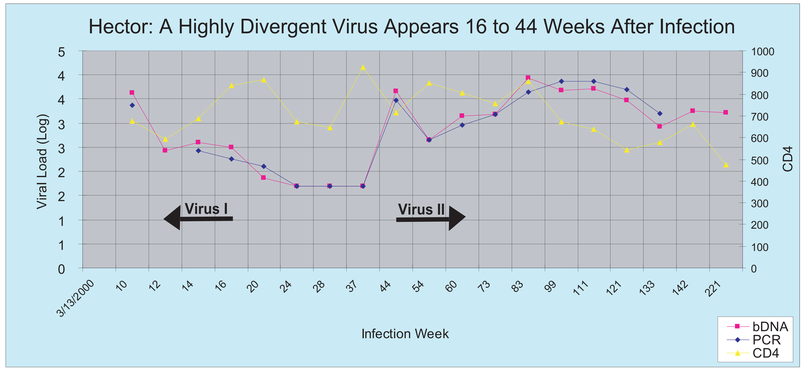
Phylogenetic Screening of 104 Recently Infected Individuals at 2 Timepoints Reveals Several Possible Sequentially Expressed Dual Infections (SEDIs)

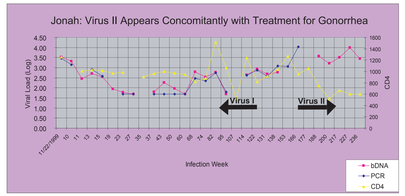


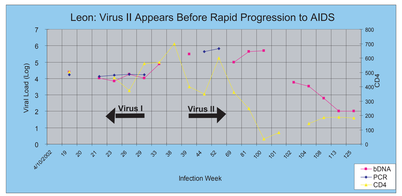
Figures 2 - 6

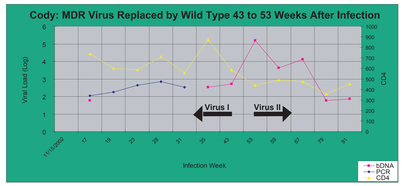
Phylogenetic and Clinical Analysis of SEDIs Reveals that the Outgrowth of a Highly Divergent Virus Is Associated with Disruptions in Markers of Clinical Progression

Note: The cases presented here are identified by pseudonyms.









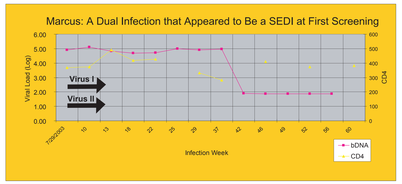
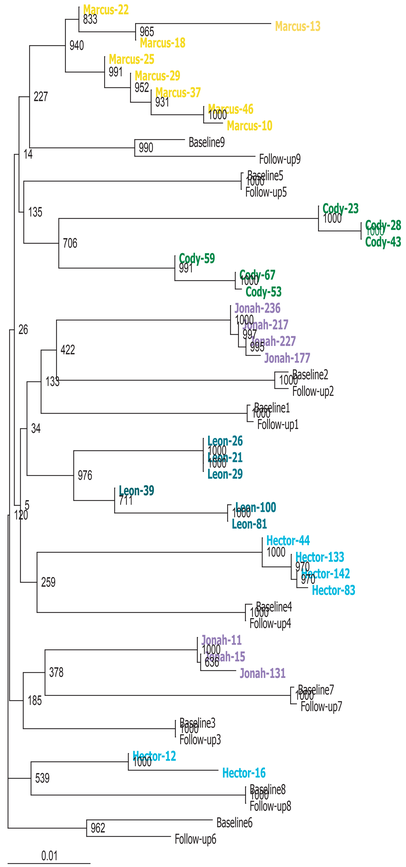


Figure 7

Analysis of Population *pol* Sequences at Multiple Timepoints Confirms 4 SEDIs and 1 Persistent Dual Infection



Conclusion

The predominance of superinfection cases among recent seroconverters may reflect a window period of susceptibility to acquiring new infections as predicted from non-human primate research. Alternatively, recent seroconverters may harbor several viruses that appear sequentially due to viral escape from immune responses.

Previously, we have found no evidence of superinfection in 56 person-years of well-characterized exposure to a genetically distinguishable virus (Grant *et al.*, BKK 2004). This time period involved 5093 exposures during unprotected anal or vaginal intercourse. Cases of superinfection among untreated, chronically infected individuals have not been published, and case series involving more than 1200 person-years of observation did not show evidence of superinfection (Gonzales 2003, Tsui 2004).

Using identical virological methods, we have now identified 4 recently infected persons who have sequentially expressed dual infections (SEDIs). Virological analysis using HMA and inspection of electropherograms indicates no evidence of the subsequent virus in the baseline specimens. These virological methods have limited sensitivity (1.5 to 3.0%) for detection of minor sequence variants. We will attempt to recruit source partners, which are required to establish whether the dual infections arose from sequential exposure and superinfection.

Studies of mechanisms blocking HIV-1 superinfection are warranted to better understand why superinfection among chronically infected humans is rare. These mechanisms may include specific anti-viral immune responses to the partner's virus, viral interference, or non-specific immune responses.

One case described here would have met criteria that other groups use for "superinfection" in that a virus population at baseline was replaced by a second highly divergent viral variant. However, analysis of the screening sample indicated that the subsequent virus was present at baseline. This case demonstrates how the dynamics of dual infection can lead to the appearance of superinfection.

Acquisition of nnRTI-resistant HIV-1 by apparent superinfection likely reflects its high prevalence in the community, relatively preserved replication capacity, and relatively high viral load.

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