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HIV-1 superinfection is associated with accelerated viral load increase but has limited impact on disease progression

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Abstract

Objective—HIV-1 superinfection occurs frequently in high-risk populations, but its clinical consequences remain poorly characterized. We undertook this study to determine the impact of HIV-1 superinfection on disease progression.

Design/methods—In the largest prospective cohort study of superinfection to date, we compared measures of HIV-1 progression in women who acquired superinfection with those who did not. Clinical and laboratory data were collected at quarterly intervals. Linear mixed effects models were used to compare post-acute viral load and CD4 T-cell counts over time in singly infected and superinfected women. Cox proportional hazards analysis was used to determine the effect of superinfection on time to clinical progression (CD4 < 200, ART initiation or death).

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Results—Among 144 women, 21 of whom acquired superinfection during follow-up, the rate of viral load increase was higher in superinfected than singly infected women (p=0.0008). In adjusted analysis, superinfected women had lower baseline viral load before superinfection (p=0.05) and a trend for increased viral load at superinfection acquisition (p=0.09). We also observed a borderline association of superinfection with accelerated CD4 decline (p=0.06). However, there was no significant difference in time to clinical progression events.

Conclusions—These data suggest that superinfection is associated with accelerated progression in laboratory measures of HIV-1 disease, but has limited impact on the occurrence of clinical events. Our observation that superinfected individuals have lower baseline viral load prior to superinfection suggests there may be host or viral determinants of susceptibility to superinfection.

Keywords

HIV-1; women; superinfection; progression; pathogenesis

Introduction

HIV-1 superinfection is defined as reinfection with a different viral variant at a later time. It has been shown to occur at an appreciable rate in several cohorts [1–3], but whether superinfection accelerates disease course remains unclear. Such an effect has been hypothesized based on two main observations. First, the association of early viral diversity with accelerated disease progression [4] suggests that viral diversity resulting from superinfection might have the same effect. Second, initial case reports of superinfection described increases in viral load (VL) at the time of superinfection detection [5,6], though whether this effect is generalizable or sustained is unknown.

Early studies investigated clinical progression in dual infection, including both coinfection (simultaneous acquisition of two variants) and superinfection (sequential acquisition). One study reported much faster development of AIDS in five dually infected than 57 singly infected individuals [7], raising concern than superinfection might have similarly severe consequences. Two small cohort studies have investigated the clinical impact of superinfection alone, rather than in aggregate with coinfection. One, analyzing 6 superinfection cases and 27 single infections, reported faster CD4+ T-cell decline in superinfected individuals [8]; the other, analyzing 7 cases and 18 single infections, reported faster VL increase in superinfected individuals [9]. These findings are consistent with the idea that superinfection accelerates progression, but are limited by small sample sizes. Both studies focused on men who have sex with men infected with subtype B virus and superinfected early in infection, so their relevance to other populations, viral subtypes and superinfection timing is unknown. Moreover, it remains unclear whether the reported differences between superinfected and singly infected individuals are a consequence of superinfection acquisition, or established prior to superinfection.

In a recent screen of 146 women from a well-characterized prospective cohort in Mombasa, Kenya, we identified and specified the timing of 21 cases of superinfection, the largest cohort of superinfected individuals published to date [1,10–12]. The incidence of superinfection in this cohort was approximately half that of initial infection. Women were

infected with viruses of subtypes A, C and D. Both intrasubtype and intersubtype superinfections were detected, with timing ranging from 63 to 1895 days post-infection. In the present study, we took advantage of frequent monitoring of this large cohort to investigate the impact of superinfection on measures of clinical progression in women infected with diverse viral subtypes. We present analyses of longitudinal change in VL and CD4 count, and time to clinical events during single infection and superinfection.

Methods

Study participants and clinical data

This study received ethical approval from the University of Nairobi, the University of Washington and Fred Hutchinson Cancer Research Center. All participants provided written informed consent. HIV-negative female sex workers in Mombasa, Kenya, enrolled in a prospective cohort and attended monthly visits, at which interviews and clinical examinations were conducted. Plasma VL and CD4 counts were quantified quarterly. HIV-1 infection timing was estimated based on a combination of serology and VL testing [13]. Superinfection timing was defined as the midpoint of the interval between the last singly infected and first superinfected timepoints [1,10–12]. HLA-B typing was performed using the LABType SSO medium resolution typing test (One Lambda); cases of HLA-B*35 were further classified by sequencing of exons 2–3 of HLA-B.

Statistical analysis

Statistical analysis was performed using R. Linear mixed effects (LME) models were run using the package *nlme*. We included all VL and CD4 data from women with at least one measurement ≥6 months post-infection and within the period in which they had been screened for superinfection. To exclude non-linear changes in VL and CD4 during acute infection, data from the first 6 months after initial infection were censored; this cutoff was determined following visual inspection of longitudinal VL data from 50 representative women to identify the time at which VL slopes flattened. Log-transformed VL and square-root-transformed CD4 data were entered into LME models to estimate the $\log_{10}$ VL and √CD4 intercepts and their rates of change over time.

Time to disease progression was evaluated by Cox proportional hazards regression, with superinfection as a time-dependent covariate. Disease progression was defined as the first of CD4<200, antiretroviral therapy (ART) initiation, or death. Follow-up time was censored at the last visit for which superinfection screening was performed.

We included viral subtype based on *env* sequences [1,10–12], genital ulcer disease at the time of infection [13], and presence of HLA-B alleles that influence HIV-1 disease progression (HLA-B*57, B*27, B*35-Px) [14] as adjustments in the LME and Cox models.

Results

Between 1993 and 2008, 309 women in the Mombasa cohort acquired HIV-1 infection and 146 of them were screened for superinfection [1,10–12]. Of these, 144 women were selected for inclusion in the present analysis, based on having initial infection timing defined to a

window of within one year. Twenty-one women acquired superinfection. All had one or more VL measurements after acute infection (≥6 months post-infection) and prior to ART, with a median of 10 measurements each. One-hundred and thirty-three women (18 superinfection cases) had one or more CD4 measurements ≥6 months post-infection and prior to ART, with a median of 10 measurements each. Baseline characteristics were similar in singly infected and superinfected women (Supplementary Table S1).

The VL intercept at 6 months post-infection estimated by LME across all women was 4.45 (95% confidence interval [CI] 4.32 to 4.57) $\log_{10}$ copies/ml, and the rate of VL change was an increase of 0.008 (95% CI 0.006 to 0.010) $\log_{10}$ copies/ml/month. The estimated √CD4 count intercept was 23.4 (95% CI 22.4 to 24.3) √CD4+ cells/mm^3, and the rate of change was a decrease of 0.085 (95% CI −0.102 to −0.069) √CD4+ cells/mm^3/month.

Results of analyses comparing VL and CD4 in women who remained singly infected throughout follow-up and women who ultimately acquired superinfection are summarized in Table 1 and Figure 1A–B. Superinfection cases were found to have 0.009 $\log_{10}$ HIV copies/ml/month faster VL increase (p=0.0008) and showed a trend for faster CD4 decline than singly infected women, by 0.047 √CD4+ cell/mm^3/month (p=0.06). Inclusion in the model of factors that influence disease progression – presence of genital ulcer disease at HIV-1 acquisition, initial viral subtype, and relevant HLA-B alleles – had negligible effect on model parameters (Table 1).

In order to determine whether superinfection cases differed from singly infected women prior to superinfection acquisition, we fit LME models to VL and CD4, including initial infection data only (Table 1). Data from all 123 singly infected women and 12 superinfection cases with one or more pre-superinfection data points were included in the VL analysis. The model showed a borderline association between ultimate superinfection acquisition and lower pre-superinfection VL intercept (−0.44 $\log_{10}$ copies/ml, p=0.06); this association reached significance in the adjusted analysis (−0.45 $\log_{10}$ copies/ml, p=0.05). No significant differences in pre-superinfection CD4 intercept or rate of change were found between non-cases (n=115) and ultimate cases (n=5), although data were limited for this analysis (Supplementary Figure S1).

In order to investigate whether VL and CD4 trajectories changed upon acquisition of superinfection, data from superinfection cases only were analyzed, comparing data pre- versus post-superinfection (Table 1). This analysis included twelve superinfection cases with VL data both before and after superinfection. A borderline association was detected for higher VL intercept following superinfection (+0.21 $\log_{10}$ HIV copies/ml, p=0.09). No significant differences were observed in pre-superinfection (n=5) and post-superinfection (n=18) CD4, although data were limited for this analysis (Supplementary Figure S1).

We next examined the impact of superinfection on time to clinical progression events. During the course of follow-up, 91 of 144 women experienced a clinical event (summarized in Figure 1C). No statistically significant effect of superinfection on time to clinical events was detected by Cox regression (HR 1.07, 95% CI 0.60–1.89, p=0.76). Adjustment for

setpoint VL, initial virus subtype and relevant HLA alleles had negligible effect on model parameters (data not shown).

Discussion

Here we present the largest study to date of the effect of HIV-1 superinfection on disease progression, comparing 123 singly infected and 21 superinfected women. We found that superinfected individuals showed significantly accelerated VL increase over time and a trend for accelerated CD4 decline. Our findings suggest that superinfection, like dual infection, has some detrimental effects on laboratory markers of disease progression. However, in contrast to results reported in an early study of dual infection [7], we did not detect a significant effect of superinfection on time to clinical events. This difference may indicate that the consequences of superinfection are modest compared to those of coinfection, or that they differ between men (who made up 4 of the 5 dually infected individuals described in [7]) and women.

Our findings of accelerated VL increase and CD4 decline in women with inter- and intrasubtype superinfections occurring up to 5 years post-infection are supported by previous smaller studies of men infected with subtype B virus and superinfected within the first year of infection [8,9]. Moreover, the larger sample size, detailed covariate data and varied superinfection timing in this cohort enabled us to perform analyses of different measures of disease progression, including analyses restricted to before or after superinfection. Interestingly, these analyses showed effects of superinfection on VL both before and after superinfection acquisition. Prior to superinfection, we observed lower VL among individuals who ultimately acquired superinfection than in those who did not. One interpretation of this finding is that lower replication by the initial virus may predispose to superinfection – possibly due to limited immune stimulation, or because a less fit virus can be more readily out-competed by a superinfecting variant. After superinfection, we observed a borderline association with increased VL intercept, suggesting that acquisition of superinfection may raise VL. While our power to detect significant differences may have been limited by sample size in some analyses, in aggregate these findings suggest that superinfection is associated with increased viral replication. Furthermore, they raise the possibility that this may be mediated by the combination of lower starting VL in individuals susceptible to superinfection, and VL increase at superinfection acquisition.

Despite observing accelerated VL increase and a borderline association with accelerated CD4 decline in superinfected women, we found no significant effect of superinfection on time to clinical events (CD4<200, ART initiation or death). This may indicate that the magnitudes of the differences in VL and CD4 – our LME models predict that a superinfected individual would have VL 0.23 $\log_{10}$ copies/ml higher and CD4 count 27 cells/mm^3 lower after 5 years of infection – are insufficient to cause detectable clinical changes. Our study of 144 women with 91 clinical events had 80% power to detect a HR of 1.82 by Cox regression; it remains possible that superinfection has a subtle effect on disease progression that we were underpowered to detect.

Overall, our findings suggest that superinfection is associated with a modest increase in VL, but no large difference in clinical outcome. Furthermore, our finding that individuals who ultimately acquire superinfection show lower baseline VL suggests there may be host or viral determinants of susceptibility to superinfection. Elucidation of these factors may shed light on early events in HIV-1 acquisition and potential avenues to its prevention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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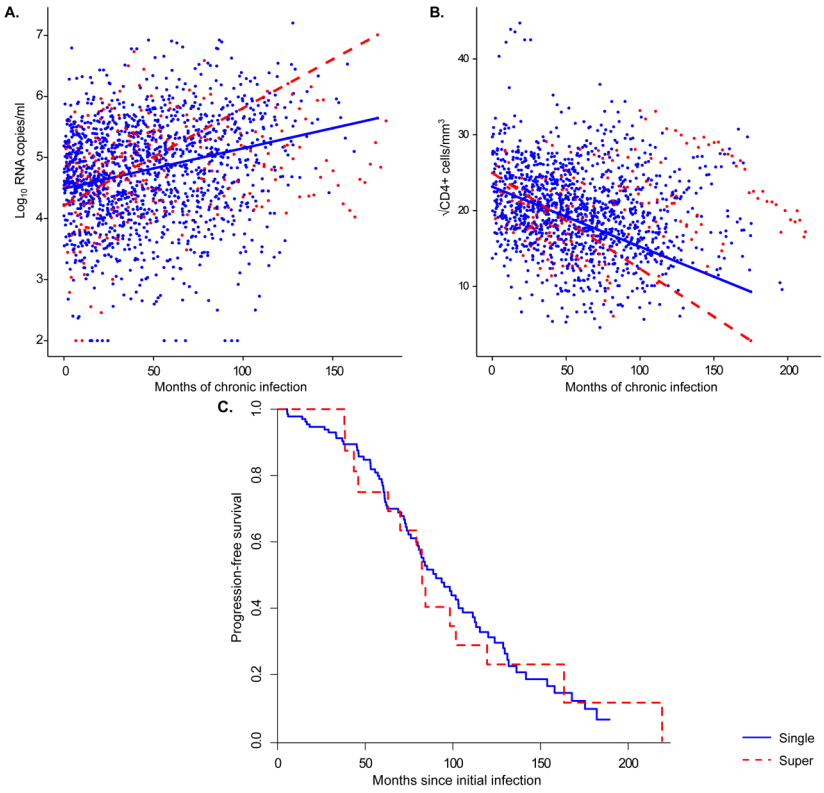

Figure 1.

Effect of superinfection on viral load, CD4 and clinical events. (A–B) Longitudinal log-transformed viral load (A) and square-root-transformed CD4 counts (B) in women who remained singly infected (solid blue) and women who acquired superinfection (dashed red). Raw data are represented by points, linear mixed model fits are represented by lines. (C) Kaplan Meier curve showing clinical progression events over time. Event-free survival is plotted against months of infection for women during single infection (solid blue) or after superinfection (dashed red).

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Table 1

Linear mixed effects model parameters

	Number of women	VL intercept (95% CI), log_{10} RNA copies/ml	p	Rate of VL change (95% CI), log_{10} RNA copies/ml/ month	p	Number of women	CD4 intercept (95% CI), √CD4+ cells/mm^3	p	Rate of CD4 change (95% CI), √CD4+ cells/mm^3/month	p
Overall: case vs. non-case										
Unadjusted	144					133				
Singly infected	123	Reference		Reference		115	Reference		Reference	
Ultimately superinfected	21	−0.28 (−0.63 to 0.07)	0.12	0.009 (0.004 to 0.015)	0.0008	18	1.82 (−0.94 to 4.58)	0.20	−0.047 (−0.096 to 0.001)	0.06
Adjusted*	127					116				
Singly infected	106	Reference		Reference		98	Reference		Reference	
Ultimately superinfected	21	−0.26 (−0.60 to 0.09)	0.15	0.010 (0.004 to 0.016)	0.0007	18	1.50 (−1.35 to 4.34)	0.31	−0.042 (−0.091 to 0.007)	0.10
Initial infection only: case vs. non-case										
Unadjusted	135					120				
Singly infected	123	Reference		Reference		115	Reference		Reference	
Ultimately superinfected	12	−0.44 (−0.90 to 0.01)	0.06	0.004 (−0.010 to 0.017)	0.61	5	1.48 (−4.29 to 7.24)	0.62	−0.025 (−0.185 to 0.134)	0.78
Adjusted*	118					103				
Singly infected	106	Reference		Reference		98	Reference		Reference	
Ultimately superinfected	12	−0.45 (−0.90 to 0.00)	0.05	0.005 (−0.009 to 0.018)	0.50	5	1.42 (−4.68 to 7.52)	0.65	−0.045 (−0.211 to 0.121)	0.59
Cases only: pre vs. post superinfection										
Unadjusted	21					18				
Pre-SI	12	Reference		Reference		5	Reference		Reference	
Post-SI	21	0.21 (−0.03 to 0.46)	0.09	−0.004 (−0.011 to 0.003)	0.27	18	−0.03 (−3.88 to 3.81)	0.99	0.065 (−0.034 to 0.164)	0.20
Adjusted*	21					18				
Pre-SI	12	Reference		Reference		5	Reference		Reference	
Post-SI	21	0.22 (−0.03 to 0.46)	0.09	−0.004 (−0.012 to 0.003)	0.23	18	0.06 (−4.01 to 4.14)	0.98	0.069 (−0.032 to 0.169)	0.18

*adjusted for initial viral subtype, genital ulcer disease at initial infection, and possession of HLAB*27, HLAB*57 or HLAB*35-Px