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BRIEF REPORT

Dynamics of Human Immunodeficiency Virus-1 Genetic Diversification During Acute Infection

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We analyzed human immunodeficiency virus envelope diversity in 98 acute infections. The within-host genetic diversity, divergence from transmitted/founder (T/F) strain, and the observed frequency of multiple T/F infections increased with Fiebig stage. These data identify rapid viral dynamics during acute infection with implications for clinical trials conducted in this setting.

Keywords. acute HIV infection; Fiebig stage; genetic diversity; HIV.

Mucosal human immunodeficiency virus (HIV) transmission is associated with a genetic bottleneck, with the majority of clinical infections established by a single transmitted/founder (T/F) virus [1]. The time shortly after HIV transmission may represent a unique window of viral vulnerability that could be targeted by vaccine-induced immunity and/or therapeutics. Therefore, understanding the pattern of viral dynamics within this critical period of time may provide valuable insights into vaccine and therapeutics development. Although it has been demonstrated that the within-host viral diversity is usually low during acute HIV infection due to the transmission bottleneck, few studies have been performed to visualize the dynamics of viral diversification within this short period of time, mainly due to the limited number of participants available at the earliest stages of infection.

In this study, we leveraged a unique opportunity of the RV254/SEARCH010 cohort, which enrolled a large number of volunteers during acute HIV infection (AHI) at different Fiebig stages [2], to investigate the dynamics of HIV-1 genetic diversification during the first few weeks of infection alongside the sequential emergence of viral markers [3].

METHODS

Patient Consent Statement

Study participants were from the RV254/SEARCH010 cohort (clinicaltrials.gov NCT00796146), a prospective study of participants with AHI in Bangkok, Thailand [2]. Most were male (96.0%) and men who have sex with men ([MSM] 92.1%) [4]. The estimated time of HIV exposure was obtained from each participant through self-report. The plasma samples were from the first available time point after HIV diagnosis before antiretroviral therapy (ART). Human immunodeficiency virus-1 subtyping was performed using a multiregion hybridization assay (MHAbce) or a multiregion subtype-specific polymerase chain reaction assay (MSS Pbce) as previously described [5, 6]. We focused on CRF01_AE infections, the predominant clade in this cohort (81.6%) [4] that represents the majority of circulating strains in Thailand [7]. A total of 353 CRF01_AE infections at Fiebig I to IV were identified, among which 98 had sequences retrieved by single genome amplification (SGA) available for genetic analysis. The study was approved by institutional review boards of Chulalongkorn University, Bangkok, Thailand and Walter Reed Army Institute of Research, Silver Spring, Maryland. All participants provided written informed consent.

Single Genome Amplification

Human immunodeficiency virus-1 sequences were retrieved from plasma samples using SGA as previously described [1]. Viral genomes were amplified as near-full length genome or 2 half genomes with approximately 1.5 kb overlap. For participants with viral load (VL) below 5000 copies/mL, the gp160 region was amplified. Between 10 and 30 amplicons (mean = 11) were obtained for each participant.

Genetic Analysis

Single genome amplification-derived envelope (*env*) sequences were codon-aligned by Gene Cutter in the Los Alamos HIV Sequence Database, followed by manual edit to obtain the optimal alignment. Aligned sequences from each participant were then analyzed using the Poisson-Fitter tool (https://www.hiv.

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lanl.gov/content/sequence/POISSON_FITTER/pfitter.html) to determine the multiplicity of infection. Single T/F infection was determined by analyzing whether sequences from an infected individual exhibited Poisson distribution of mutations and star-like phylogeny after hypermutations with APOBEC3G/F signatures were excluded. Multiple T/F infection was determined when sequences from an individual deviated from both Poisson distribution of mutations and star-like phylogeny when positions with APOBEC mutations were excluded. Transmission of multiple T/Fs was further confirmed by visual inspection of the highlighter plot, which showed distinct viral lineages with an interlineage distance higher than the maximum achievable distance given their Fiebig stage [1]. For participants who had overall low sequence diversity but violated star-like phylogeny after excluding APOBEC mutations, we determined that they were most likely infected by highly similar variants, although the possibility of early host selection could not be excluded.

For single T/F infections, the T/F sequence was inferred as the consensus sequence of the alignment. The within-host genetic diversity and divergence from the T/F strain were calculated using the MEGA6 software with K2P model as previously described [8].

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 8 software.

RESULTS

A total of 353 acute CRF01_AE infections at Fiebig I through IV were identified in the RV254/SEARCH010 cohort (median days from exposure = 19). Analysis of VL dynamics showed that the median VL was lowest at Fiebig I (1.63×10^4 copies/mL), increased dramatically at Fiebig II (1.03×10^6 copies/mL), highest at Fiebig III (2.83×10^6 copies/mL), and dropped to a lower level at Fiebig IV (5.63×10^5 copies/mL) ($P < .001$; analysis of variance test) (Figure 1A).

We then investigated the dynamics pattern of viral diversification across different Fiebig stages for 98 participants with SGA sequences available (Supplementary Table 1). To delineate the dynamics of within-host sequence evolution from the T/F strain, we focused on 70 (71.4%) participants who were identified as single T/F infections (Figure 1A and B). We excluded 23 (23.5%) infections established by multiple T/F viruses (Figure 1B) and 5 infections (5.1%) likely to be established by highly similar variants (Supplementary Figure 1), because sequences from such cases were evolving from multiple ancestors and the frequent interlineage recombination could confound the analysis [8].

Among the 70 single T/F infections, the within-host mean *env* diversity increased gradually with Fiebig stage (median: 0.023% at Fiebig I, 0.035% at Fiebig II, 0.036% at Fiebig III, and 0.050% at Fiebig IV) (Figure 1A). Similar dynamics were observed for the mean *env* divergence (Figure 1A). The extent of viral diversification from Fiebig I to IV was statistically significant ($P = .004$ for diversity, $P = .01$ for divergence; Mann-Whitney test). There was no observable increase in either *env* diversity or divergence from Fiebig II to III, possibly reflecting a relatively rapid transition from Fiebig II to III (3.7–7.7 days) [3]. Both the within-host *env* diversity and divergence tended to increase with the estimated days from exposure (Figure 1C and Supplementary Figure 2).

Twenty-three (23.5%) participants were determined to have multiple T/F infections (Figure 1D). All were MSM except for 1 heterosexual female (Supplementary Table 1). It is interesting to note that when this frequency was stratified by Fiebig stage, the observed frequency of multiple T/F infection increased dramatically with Fiebig stage (Figure 1D). Only a single participant was detected as multiple infection in Fiebig I (7%). The frequency climbed to 40% in Fiebig IV ($P = .048$; logistic regression) (Figure 1D).

DISCUSSION

The relatively large number of participants enrolled in the RV254 cohort provides a unique opportunity to visualize the dynamics of HIV-1 evolution within the narrow window of acute infection at a population level. Although the general VL pattern at Fiebig stages I through IV observed here was highly similar as previously reported [3], the median VL level in the RV254 cohort was consistently higher at each Fiebig stage than previously observed. Further investigations are needed to understand whether this difference reflects an overall higher replication capacity of CRF01_AE viruses or is due to host factors. To our knowledge, this is the largest number of participants identified at the Fiebig I, which made it possible to determine the degree of viral diversity at the earliest stages of HIV infection. Our data show relatively low degree of viral diversification in Fiebig I, which is in line with a significantly smaller reservoir size at Fiebig I compared with later Fiebig stages [9]. Despite the relatively low level of viral evolution, participants in Fiebig I exhibited a considerable level of variability in terms of both sequence diversity and divergence. This could indicate either a highly variable duration of the eclipse phase from individual to individual or a diverse replication rate of T/F viruses transmitted to different individuals.

The overall prevalence of multiple T/F infections identified here is nearly the same as previously reported for acute/early subtype B infections (mainly through the heterosexual route) [1]. Nevertheless, stratifying the data by Fiebig stage revealed a dramatic increase in the observed frequency of multiple infections over time. This observation suggests differential

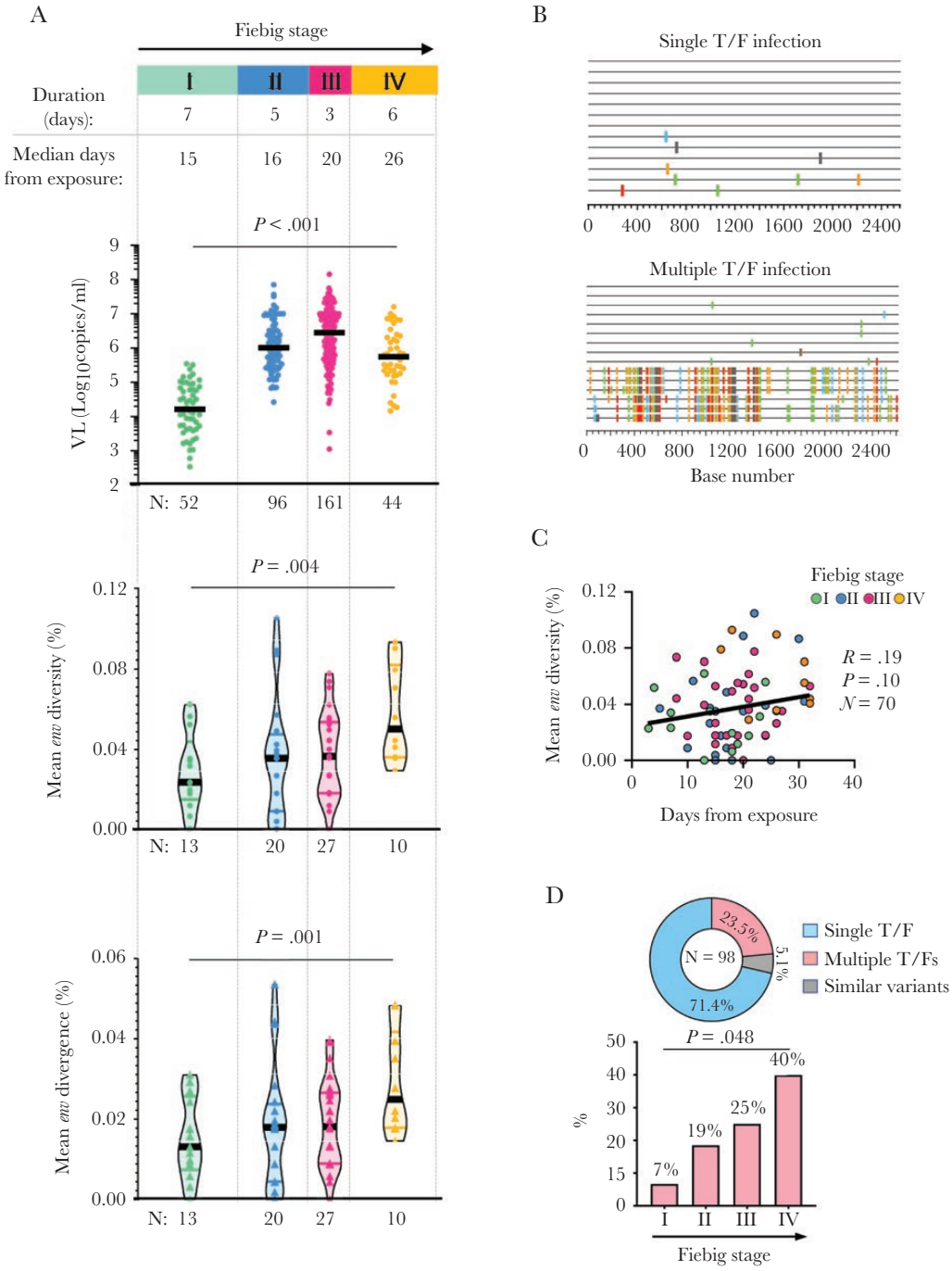

Figure 1. (A) Dynamics of viral load (VL), mean within-host *env* diversity and divergence at Fiebig I through IV. The estimated duration of each Fiebig stage and the median days from presumed human immunodeficiency virus exposure are shown. The black lines show the median values. (B) Highlighter plots show 1 example of single transmitted/founder (T/F) infection and 1 example of multiple T/F infection. (C) Correlation between mean *env* diversity and estimated days from exposure. Participants in different Fiebig stages are color-coded. (D) Frequencies of single and multiple T/F infections among 98 sequenced participants and the proportion of multiple T/F infections detected in each Fiebig stage.

replication rates among multiple T/F variants limiting the potential to sample these in earlier Fiebig stages. This is supported by previous observations in the RV217 cohort [10]. In 4 of 5 multiple infections, the minority variants that were not detected by SGA at the earliest time points were detectable by deep sequencing. Therefore, the increased frequency of heterogenous infections observed here is more likely reflecting differential replication rates of multiple variants, although early superinfections could also contribute. Further investigations by deep sequencing the samples from early Fiebig stages will help to determine the likelihood of each scenario.

CONCLUSIONS

In summary, the current study uncovered the rapid dynamics of HIV-1 genetic diversification within the first month of infection with several clinical implications. First, it provides useful viral genetic reference at different AHI stages for future clinical intervention trials in AHI. Second, for infections established by multiple T/F viruses (which could be as high as 40% in this cohort as observed in Fiebig IV), early ART in Fiebig I could limit the dissemination of the "slower" T/F lineages in both the reservoir and blood, thereby minimizing the chance of interlineage recombination, which substantially increases viral diversity and facilitates viral immune escape [8]. Although multiple clinical benefits of early ART, including smaller reservoir size [9], lower frequency of VL blip [11], and more intact immune system [12] have been characterized, the current study identifies additional potential benefit from a viral genetic perspective.

Supplementary Data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Potential conflicts of interest. J. A. previously received honoraria for participating in advisory meetings for ViiV Healthcare, Merck, Gilead, Roche, and AbbVie. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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