Sequence Note

Full-Length HIV Type 1 Genome Analysis Showing Evidence for HIV Type 1 Transmission from a Nonprogressor to Two Recipients Who Progressed to AIDS

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ABSTRACT

Epidemiologically-linked HIV-1 transmission cohorts serve as excellent models to study HIV disease progression. The actual relationship between viral variability and HIV disease outcome can be extrapolated only through such rare epidemiologically linked HIV-1-infected cohorts. We present here a cohort of three patients with the source termed donor A (a nonprogressor) and two recipients B and C. Both recipients acquired HIV through blood transfusion from donor A and have progressed to AIDS. By analyzing 15 near full-length HIV-1 genomes (8.7 kb each genome) from longitudinally collected peripheral blood cell samples (four time points for patient A, four for patient B, and seven from patient C), we were able to demonstrate transmission of HIV from donor A and epidemiologic linkage among members A, B, and C after 10 years of HIV infection. These analyses are novel in demonstrating that HIV-1-infected nonprogressing individuals bear the potential to transmit HIV-1 variants and that HIV variants, which led to a benign disease in a nonprogressor donor, were able to cause disease in other individuals. Overall, these studies highlight the utility of full genome sequencing in establishing epidemiologic linkage in a chronically infected HIV cohort after 10 years of initial infection.

INTRODUCTION

THE RATE OF HIV DISEASE PROGRESSION varies greatly among infected individuals, which is defined invariably by increasing plasma viral loads and concomitant decline in the CD4 T cell counts. A small but rare subset of chronically infected, therapy-naive individuals appears to maintain high and stable CD4 and CD8 T cell counts and low to undetectable plasma viral loads for an indefinite number of years. As a result, no viral amplification and viral isolation in vitro can be achieved from plasma of such individuals.1,2 These individuals are termed long-term nonprogressors (LTNPs). Some of these nonprogressing individuals harbor fewer than 10 copies of proviral DNA/ml blood and show strong immune responses1,4 and high secretion of CD8 antiviral factor(s) (CAF).3,5 Additionally, in some extremely rare cases there is a complete absence of viral evolution over time in such individuals.6 Although such individuals remain healthy throughout the course of infection with below detection viremia, in many cases therapy naive, their ability to transmit HIV to other healthy individuals remains unknown. Further, it remains unclear if the HIV-1 strains, which cause a benign disease in nonprogressors, can actually cause disease progression in other individuals upon transmission.

By using the well-described approaches of both Lookback and Traceback, clusters of distant HIV transmissions can be identified.7 In this study, we describe detailed longitudinal analyses using full-length HIV-1 genome sequence analyses from one such cohort comprised of three HIV-infected individuals (a nonprogressing donor A and two recipients B and
C). The donor A, who likely acquired infection in 1982, and has remained healthy maintaining nonprogressive status with high CD4+ and CD8+ T cell counts (700 and 900, respectively, cells per mm³) and with < 425 HIV-1 copies/ml of plasma. The two recipients were infected in autumn 1989 (recipient B) and summer 1989 (recipient C), respectively, through blood transfusion from donor A. They have progressed to AIDS and were put on antiretroviral therapy. Patient B has recently died of AIDS and the CD4+ and CD8+ T cell counts of patient C remain at 7 cell/mm³. With the help of detailed full-length HIV-1 genomes, we investigated viral evolution, epidemiologic linkage, host-selective forces, and correlation of HLA type on HIV disease progression and nonprogression. The rarity of such cohorts accents their existence as invaluable models for understanding HIV epidemiology and also in delineating the role of host and viral factors in HIV pathogenesis.

Gene-Amp XL PCR kit (Perkin-Elmer, Emerville CA) was used to amplify near full-length HIV genomes (8766 base pairs, the LTR domains were amplified separately) as previously published. A total of four longitudinal cohort samples obtained from donor A were termed A1, A3, A5, and A6 and corresponded to years 1996, 1998, 1999, and 2000. Similarity four time points from patient B were termed B3, B4, B5, and B6 and corresponded to years 1997, 1998, 1998, and 1999 for sample collection, with C2, C3, C5, C6, C8, C10, and C11 representing patient C samples obtained from 1992, 1994, 1992, 1993, 1996, 1998, and 1999.

To investigate the presence of patient mutations within a known cytotoxic T lymphocyte (CTL) epitope, a database search was conducted within the Los Alamos (NM) immunology database. Class I HLA typing was performed with the Dyonal Reli Sequence Specific Oligonucleotides (SSO) kit (Dynal Biotech, Oslo, Norway). DNA extracted from blood collected in VACUTAINER ACD-A (yellow-top; Becton Dickinson) is used in PCR target amplification. The amplified products were hybridized to specific oligonucleotide probes and the probe-bound amplified product was detected by color formation. In HLA-A, B and Cw assays the specific target DNA sequences are the polymorphic second and third exons of the HLA class I genes.

Nucleotide sequences and peptide sequences were aligned using CLUSTAL W 9 and manually edited in Se-Al according to their reading frame. The best-fitting nucleotide-substitution model was selected using Modeltestv3.06. Phylogenetic trees were reconstructed in PAUP4:0b10, starting from a neighbor-joining tree under a heuristic maximum likelihood search that implemented both nearest-neighbor interchange (NNI) and subtree pruning-regrafting (SPR). Bootstrap analysis was performed using the neighbor-joining method on 1000 replicates. Bayesian trees were reconstructed in mrbayes v2.01. Network analysis was performed in Splitstree 2.4.

Successful amplification of near full-length HIV-1 genomes was achieved from a total of 15 peripheral blood mononuclear cell (PBMC) patient samples collected between 1992 and 2000 from all three cohort members A, B, and C.

The most interesting and unique feature of the nef gene was the possible presence of two variants in donor A, who has remained nonprogressive throughout the course of this study. Three of four sequences from patient A showed a 14 amino acid insertion between residues 16 and 29 (Fig. 1). Only one sequence (Nef A1) (Fig. 1) did not show any insertion coinciding with the first sampling date obtained in 1996. The three
consecutive time points A3, A5, and A6 obtained from donor A all revealed the presence of a nef insertion. A careful examination showed that only the sequence without the insertion was detected in all of the sampling dates analyzed for both recipients (Fig. 1). Phylogenetic analysis also showed good concordance between these sequences from all three patients (Fig. 1). Furthermore, cloning and sequencing were conducted from all four time points from donor A confirming the presence of insertion in only the later three sampling dates (data not shown). At the early sampling point of patient A, there was an absence of this nef insertion and the donor maintained nonprogressive status. Thus despite the appearance of this insertion in later time points, the disease status of the donor remained unaffected. Because the infections were confirmed in 1987, earlier samples close to transmission were not available. Also, as a number of intermediate samples were not available the accurate timing remains to be determined for the emergence of this insertion in vivo, other than being first detected in 1998. Nonetheless, the presence of a unique nef insertion (HIV Los Alamos Database) in donor A and the transmission of a second variant to two recipients appeared likely as the variant without insertion was also observed in donor A. Due to unknown reasons, the variant with insertion remained dominant in the bloodstream. Therefore, though it is unlikely that this mutation is linked to the nonprogressive status of the donor, its functional significance in transmission remains to be elucidated.

As following transmission of HIV from a nonprogressor, the two recipients progressed to AIDS, our sequence analysis...
showed clear evidence of intrahost evolution of HIV in all the members. Since the two recipients progressed to AIDS, it was apparent that HLA type may have influenced and defined the disease course in three members of the cohort. Analysis of the effect of selectively altered sites of the HIV genome may assist in understanding the viral mechanisms involved in evading the host’s immune system through propagation of CTL escape mutants. HLA typing revealed patient A possessing an HLA type of A2, A3, B57, B65, and unknown for locus C; patient B showed to be A2, A11, B36, B62, and CW1 specific, while progressing recipient C was similarly found to be HLA A2, A24, B7, and B13 type again with an unknown C locus. Sites identified as patient HLA specific possibly correlated with the generation of CTL escape mutations, therefore patient disease progression includes those identified for patient B and C within the p17 region of the known CTL motif SLYNTVATL, where recipients B and C showed a valine substitution for threonine. They also showed a known CTL reverse transcriptase motif (peptide position 265) AIPQ/SSMKT, existing only in recipient B, where an arginine residue was substituted for a lysine. Though patient-specific CTL escape mutations have also been identified for patients A and C, we have concentrated on those occurring within patient B HLA loci, as this is the patient who has shown the fastest clinical disease leading to eventual death. Another known CTL motif within the p24 gag region of the HIV genome is ACQGVPGPHEK, at position 359. This motif also contained point mutations where glycine was consistently changed to either glutamic acid or arginine. It was unique to patient B. This suggests that such CTL-associated changes may contribute to the generation of CTL escape mutations that may aid in the evasion of the host immune response, and hence advancement of disease.

To reinforce the epidemiological linkage of the transmission chain under investigation, an exhaustive database search for related full-length HIV-1 sequences was performed using BLASTN and a maximum likelihood phylogenetic tree was re-constructed (Fig. 2). Within the HIV-1 subtype B phylogenetic tree, the cohort clearly constitutes a single cluster, supported by high bootstrap probabilities. Interestingly, the donor A lineage appears to be the outgroup for the two recipients and it was noted that recipient C revealed one long branch (Fig. 2) segregating earlier time points from samples obtained from 1996 to 1999. As this is correlated with clinical patient profile, it can be deduced that the emergence of host-induced viral variation and hence viral evolution at recent time points occurred in concert with the rapidly progressing status of AIDS patient C. This pattern was also evident through analyses obtained from all the individual genes (data not shown). It is apparent from our analyses that following transmission of HIV, the two recipients gradually deteriorated over a 15-year period to low CD4+ T+ cell counts and high viral loads despite continuing antiretroviral therapy since 1997. These data suggest a possible role in vivo viral divergence and host selection pressure over time in the transition of a virus associated with nonprogression in the donor to a virus associated with gradual progression of HIV in two recipients. Selection could also have caused the discordant phylogenetic patterns detected over time between cohort members. Differential selective pressure was found to have substantially contributed to virus evolution within these three cohort members.

Furthermore, in relation to host factors we also investigated the association of patient-specific HLA types and its correlation to disease progression. Interestingly, donor A showed the HLA B57 loci, which was shown to be associated with slow or nonprogression of HIV disease, irrespective of the mutations proposed to generate CTL escape mutations. Conversely, patients B and C have HLA B56 and B7, respectively, both of which have been associated with poor disease outcome, and hence it is possible that the HIV status of these individuals is associated with their HLA types, and is not only due to the propensity CTL escape mutations.

Overall, our study using HIV-1 full-length genomes raises the possibility that nonprogressors in some cases may harbor either pathogenic and nonpathogenic variants or the benign variants can be activated in other hosts. Thus, host genetics may act as a driving force for positive selection and divergence of infecting strains over time. Nonetheless, despite genetic drift in HIV strains in each member of the cohort, sophisticated phylogenetic tools along with full-length HIV genome sequencing over time fully supported the epidemiologic linkage in this cohort.

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REFERENCES


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