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Brief Communication

A case cluster demonstrating the relationship between HLA concordance and virologic and disease outcomes in human immunodeficiency virus infection

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ABSTRACT

We present a detailed analysis of sexual HIV transmission from one source partner to two recipients. The HLA haplotypes between the source partner and one recipient were very similar with 7 out of 8 HLA alleles from four loci (HLA A, B, C and DRB) shared, while the other recipient shared only one allele. The immunologic outcomes between the two recipients differed dramatically, despite the absence of apparent virologic differences in their inoculums. We suggest that non-viral factors, which might be related to differences in the HLA profile, played a role in determining different CD4+ T-cells dynamics for these two recipients.

Introduction

Investigating how the host immune response influences HIV evolution and disease progression is important to understand HIV pathogenesis. Among the many viral and host factors affecting HIV infection, MHC class I restricted cytotoxic T lymphocytes (CTL) are an important determinant of viral evolution and disease outcome (Ahuja et al., 2008). Mother-to-child studies have demonstrated an association between the concordance of HLA haplotypes between an infected mother and child with transmission and disease progression (Drummond et al., 2012). We describe a sexual transmission cluster in which concordant versus discordant HLA haplotypes between source and recipients were associated with considerably different disease trajectories.

Results

Source partner

The source was a 26-year-old male who reported sex with other men as an HIV risk factor. He reported having unprotected sexual intercourse with Recipients #1 and #2 during the first several months after he was presumptively infected with HIV-1. During this time, he had not yet been tested for HIV-1. He was eventually diagnosed with HIV-1 at the same time as Recipient #2. The EDI for the source was ~5–6 months before the HIV-1 transmission events to both recipients. The first seminal and blood samples were collected from the source ~6 months after the EDI. At that time the blood plasma viral load was 5.2 $\log_{10}$ HIV RNA copies/mL while seminal plasma viral load was 4.8 $\log_{10}$ copies/mL; CD4+ T-cell count was 270 cells/mm^3 (Table 1).

Recipient #1

Recipient #1 was a 24 year-old MSM who noted having receptive anal intercourse with the source partner during the previous months. He was diagnosed with HIV six months after his EDI. Initial blood plasma viral load was 4.6 $\log_{10}$ copies/mL and

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Table 1
Subjects' characteristics.

Variable	Source	Recipient #1	Recipient #2
Age at EDI, years	26	24	30
Risk factor	MSM	MSM	MSM
Number of reported sexual partners	3	1	1
Mean CD4 T cell count, cell/mm^3 [min–max]	264.7 [230–294]	294.0 [246–358]	877.6 [738–1344]
Mean CD4 T cell percentage [min–max]	19.7 [18–21]	15.6 [12–18]	38.6 [33–49]
Mean CD8 T cell count, cell/mm^3 [min–max]	654 [588–764]	1088.9 [716–1780]	1040.4 [634–1188]
Mean CD8 T cell percentage [min–max]	52.3 [51–53]	55.0 [52–60]	45.8 [34–53]
Mean blood plasma HIV RNA level, log-copies/mL [min–max]	5.03 [4.86-5.20]	4.86 [4.39–5.20]	4.93 [3.80–5.43]
Seminal plasma HIV RNA level, log-copies/mL at diagnosis	4.82	NA	NA
Viral subtype	B	B	B
EDI in days to first visit	170	188	70
Drug resistance genotype-Protease-Reverse transcriptase	-L10I – None	-L10I – None	-L10I – None
HLA alleles	A*02 (A1) A*32 (A2) B*13 (B1) B*49 (B2) C*06 (C1) C*07 (C2) DRB1*11 DRB2*13	**A*02 (A1)** A*23 (A2) **B*13 (B1) B*49 (B2) C*06 (C1) C*07 (C2) DRB1*11 DRB2*13**	A*24 (A1) A*26 (A2) B*39 (B1) B*40 (B2) C*03 (C1) **C*07 (C2)** DRB1*04 DRB2*08

EDI: Estimated Date of Infection.
* Mean within the first month for source partner and within the first 300 days for both recipient before antiretroviral treatment initiation. In bold: HLA alleles matching with source partner.

CD4+ T-cell count was 326 cells/mm^3 (Fig. 1A). HLA haplotyping revealed that Recipient #1 was a 7 out 8 match with the source at the three MHC class I HLA loci and one MHC class II (DRB) locus (Table 1).

Recipient #2

Recipient #2 was a 30 year-old MSM who also reported having unprotected receptive anal sex with the same source partner during the previous months. His EDI was two months before his diagnosis. His initial blood plasma viral load was 3.8 $\log_{10}$ copies/mL, and his initial CD4+ T-cell count was 962 cells/mm^3 (Fig. 1A). HLA haplotyping showed only a single allele match with the source (Table 1).

Disease progression

Despite the same source partner and a closely related transmitted virus (mean genetic distance of 0.008 substitution/site between *env* sequences obtained at the earliest timepoint after infection for Recipients #1 and #2), the trajectory of CD4 decline was very different between the two recipients within the first months of infection before ART initiation (started 318 and 308 days post-diagnosis respectively) (Fig. 1A). While Recipient #2 maintained a high CD4+ T cell count (nadir of CD4+ T-cells=738/mm^3), Recipient #1's CD4+ T-cell count dropped to 246 cells/mm^3 during the first 300 days of untreated HIV-1 infection. There was, however, no difference in blood plasma viral load between recipients (Fig. 1A).

Transmission origins

Using *env* sequences, both Bayesian Markov chain Monte Carlo (BMCMC) between-host phylogenetic analyses (Fig. 2) and pairwise genetic distance (*env* mean genetic distance source–recipient #1=0.003 and source–recipient #2=0.001) supported the epidemiologic history of a single source partner transmitting HIV-1 to Recipients #1 and #2. Subsequent analyses allowed a more precise characterization of the transmission event and the origin of transmitted variant(s). First, phylogenetic reconstruction (Fig. 2) for the pair of source and recipient #1 suggested that the viral population sampled from the recipient's blood plasma originated from one variant most likely originating from the source's blood plasma (posterior probability=0.62, Fig. 2). Visual inspection of tree topology for the pair of source and recipient #2 suggested multiple founder variants originating from both source semen and blood; however, with only limited branch support (posterior probability=0.47, Fig. 2). Investigation of co-receptor usage within the source and both recipients revealed a mixed population of X4 and R5 virus in the semen of the source, but only R5 in blood, while recipient #1 had only R5 virus in blood and recipient #2 had both X4 and R5 variants in blood. These observations supported the origin of the transmitted variants inferred by phylogenetic analysis (Fig. 2).

Viral evolution

The rate of evolution of HIV-1 *env* differed significantly ($p<0.001$) between the recipients despite the same source partner (Fig. 1B). Recipient #1 showed little evidence of viral divergence from baseline during the first year of infection (0.004 substitution/site), while Recipient #2 demonstrated early and high diversification of the *env* sequences (0.143 substitution/site). Longitudinal samples obtained from the recipients permitted the estimation of the rate of evolution in the viral population using BMCMC inference (Gao et al., 2001). Two different patterns of viral genetic diversification were observed (Fig. 1B), with a 3.4-fold higher median rate of evolution within *env* for Recipient #2 (7.09×10^{-5} substitution per nucleotide site per day [1.56×10^{-6} –9.47×10^{-5}]) versus Recipient #1 (2.09×10^{-5} substitution per nucleotide site per day [4.13×10^{-6} –2.61×10^{-5}]). This pattern was not seen in *gag* or *pol*/RT likely because of limited sequence variation in these genes early during infection.

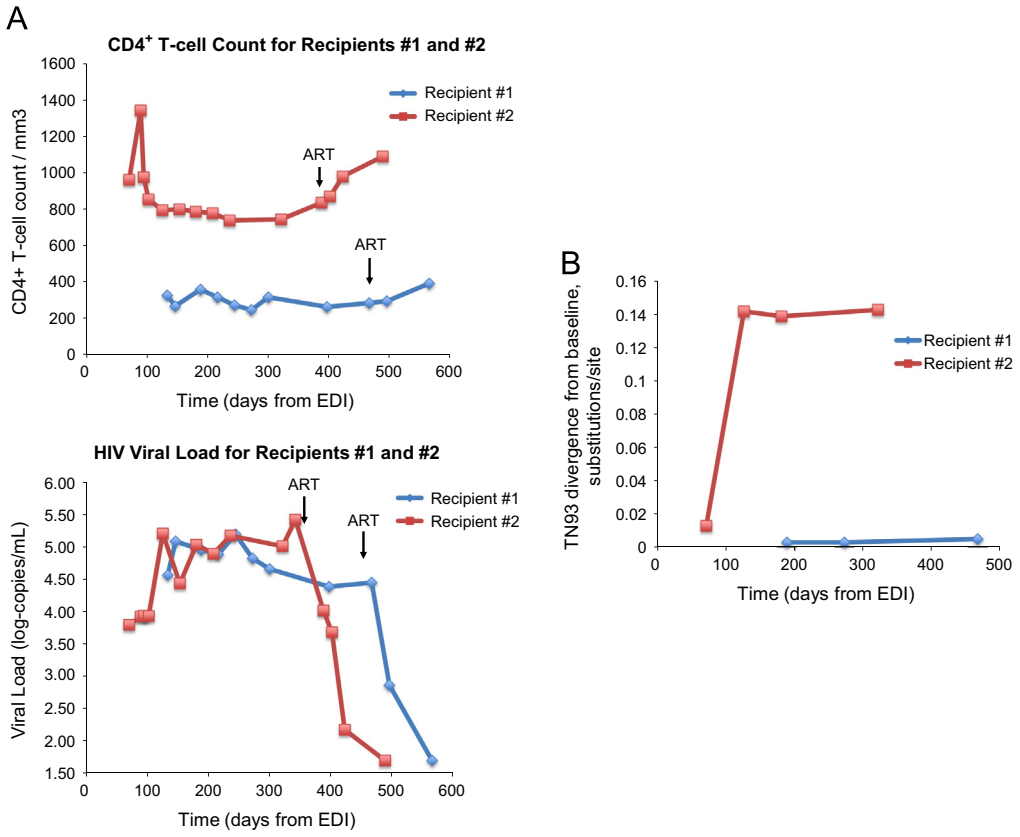

Fig. 1. (A) Comparative virological data for Recipients #1 and #2. The CD4+ counts (/mm^3) and log viral loads (copies/ml) for Recipient #1 (blue) and Recipient #2 (red) are shown. Both recipients were exposed to the same index source partner within the same period of time. They remained off therapy for 400–500 days post estimated date of infection (EDI). Whilst Recipient #1 showed a low stable CD4+ – cell count during this period, the CD4+ T-cell count for Recipient #2 remained above 800/mm^3. (B) Genetic divergence from baseline within partial *env* region for Recipients #1 and #2. Genetic distance between curated partial *env* sequences at each sampled time (nucleotide position relative to HXB2 genome start 6887-7287) relative to sequences sampled at first time-point. Initial sampling dates were respectively 188 and 70 days from estimated date of infection (EDI) for subjects B and C. Follow-up duration was respectively 279 and 351 days. During that period, both subjects remained off treatment. Genetic distances were measured under a TN93 Model as implemented in MEGA v5.

CTL epitope evolution

In this study, the source partner and Recipient #1 expressed HLA class I alleles and class II alleles at the DRB locus that were almost perfectly concordant (7/8 allele match) (Table 1), but between the same source and Recipient #2 only one HLA class I allele was matched. To investigate the hypothesis that HIV variants transmitted to Recipient #2 (but not to Recipient #1) evolved to escape new CTL selective pressure, we looked for evidence of CTL escape within sequences from the two recipients using computationally predicted changes in MHC binding scores (O'Brien et al., 2001) in relation to each recipient's HLA haplotype. In the partial *pol*-RT sequences, there were significant changes in binding affinity at several epitopes (defined as >2 fold change in the IC_{50} [nM]) (Table 2). For example, Recipient #2 was the only individual with HLA C*03, and there was a change in binding affinity for the *pol*-RT epitope (FSVPLDKEF). The viral population in the source's blood had the epitope sequence (FSVPLDKEF) and an IC_{50} of 11.12 (nM), while at the last timepoint available, Recipient #2 had the epitope sequence (FSVPLB**H**K**D**F) and an IC_{50} of 29.16 (nM) (Tables 1 and 2), suggesting viral escape from CTL pressure. Notably, the mutations were absent in the earliest sequences obtained from Recipient #2 and the observed mutations D120H and E122D were stable overtime with no evidence of reversion. The second example of probable escape was at the HLA B*40 *pol*-RT epitope (EDFRKYTAF). Only Recipient #2 had this HLA allele. The viral population in the source's blood had the epitope sequence (EDFRKYTAF) and an IC_{50} of 97 (nM), while at the last timepoint available, Recipient #2 had the epitope sequence (**D**DFRKYTAF) and an IC_{50} of 15523 (nM) (Tables 1 and 2). No evidence of escape in HLA predicted epitopes were found in recipient #1.

Discussion

We describe a transmission cluster from one source partner to two recipients. Recipient #1 shared 7 out of 8 HLA alleles with the source, while Recipient #2 shared only one. Similar to reports of mother to child transmission (Ahuja et al., 2008; Drummond et al., 2012), we observed a greater CD4 count decline in the recipient who shared the most HLA alleles with the source. To investigate possible mechanisms for this clinical discordance, we evaluated differences in transmitted variants (compartment of origin, co-receptor usage and number of transmitted variants), evolution of viral populations after transmission, and estimated changes in CTL binding affinity in putative epitopes.

Several possible differences in the transmitted variants between the two recipients were identified. Although not conclusive, phylogenetic analysis suggested that Recipient #1 was infected with a single transmitted/founder variant derived from the blood of the source, while Recipient #2 was possibly infected with multiple variants arising from the blood and semen of the source. Additionally, Recipient #1 appeared to have been infected with a pure R5-tropic variant while Recipient #2 appeared to be infected with mixed R5-X4 tropic variants. Previous larger studies have demonstrated that viral replicative capacity is a major

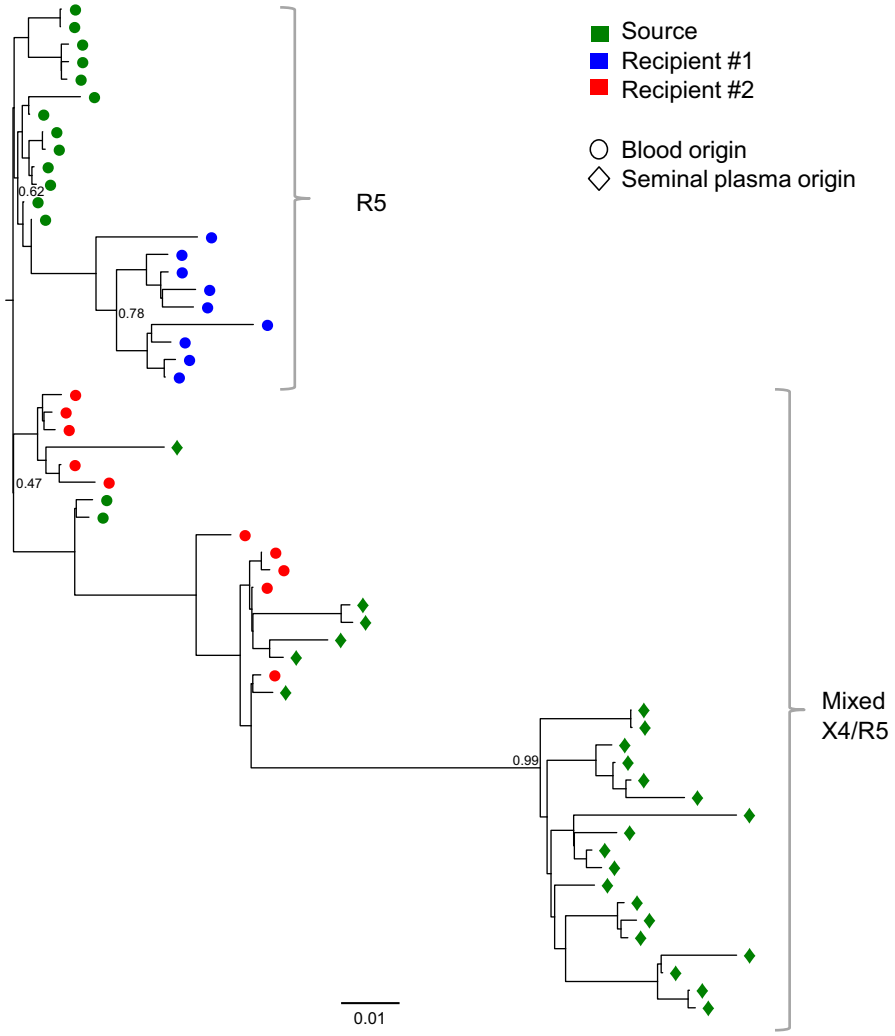

Fig. 2. Bayesian Markov Chain Monte Carlo (BMCMC) phylogenies of source and both recipient's viral population. Partial NGS *env* sequences from the index donor partner are in green, sequences from Recipient #1 and #2 are respectively in blue and red. Full dots show NGS sequences from blood plasma RNA and diamonds show NGS sequences from donor seminal plasma RNA. Scale bars represent genetic distances in substitutions/site. Posterior probability of main clusters is indicated at the root. Tropism predictions are indicated. Note that source samples were obtained after the recipients.

determinant of pathogenesis within recipients and correlates significantly with CD4+ T-cell decline (Richman et al., 2003). Infection with multiple variants, higher viral diversity and mixed tropism are also associated with a more rapid CD4 decline (Le et al., 2013). In this transmission cluster we found the opposite to be true. Recipient #2, who had evidence of a multiple variant transmission and higher viral diversity, and mixed tropism, presented with slower initial immunologic progression as demonstrated by significantly higher peripheral CD4+ T-cell counts compared to Recipient #1 (Fig. 1A). This increased viral diversity in *env* may have been due to recombination events between transmitted variants, or other factors like differences in neutralizing antibody between subjects, (not evaluated in this report). However, the lack of evidence for viral escape from CTL epitopes suggests that the differences in HLA haplotypes was not the driving force for the increased diversity seen in Recipient #2. These observations are limited by the sequence data available, and so it is conceivable that over time, the HLA haplotype differences could contribute more to viral evolution.

Previous reports showed a correlation between viral setpoint of donors and recipient (Zagordi et al., 2011). Here, we did not find any evidence for differing viral replicative capacity between the two recipients (Fig. 1A). This suggests that the two potential CTL escape mutations we observed in *pol*-RT HLA epitopes B*40 and C*03 of the HIV sampled from Recipient #2 did not affect viral replication. Given that there was only one discordant HLA allele between the source and Recipient #1, we did not find any escape mutations in that subjects HIV (Table 1). Previous studies showed that specific HLA epitopes (e.g. HLA-B*57 and HLA-B*27 alleles) are associated with slower disease progression (Prince et al., 2012; Gianella et al., 2011). However, none of the major protective HLA alleles were identified in our study. Sequence changes in other regions such as *nef* also need to be evaluated along with additional phenotypic characterization to more finely evaluate the CTL immune response.

In conclusion, we found that in this unique transmission cluster, neither transmission of multiple variants, X4 tropic virus, nor higher viral diversity was associated with a more rapid CD4+ T cell decline. These findings suggest that a non-virologic factor was associated with the differences in immunologic outcomes between the two recipients. Although HLA concordance has been associated with more rapid CD4+ T cell decline (Draenert et al., 2006), it has been demonstrated mainly through effects on virologic evolution. Here we demonstrate that despite significant virologic differences, another factor had a more significant effect on the CD4+ T-cell counts of the two recipients. Further

Table 2
Two epitopes with evidence of CTL escape within recipient #2 only.

Region (AA positions of epitope relative to protein start in HXB2)	Days post EDI (number of sequences[a])	Sequence	HLA allele of recipient
Pol-RT (116-124)	70 (3/3)	F S V P L D K E F	C3
	180 (5/5)	- - - - - - H - D -	
	321 (2/2)	- - - - - - H - D -	
Pol-RT (122-130)	70 (3/3)	K E F R K Y T A F	B40
	180 (5/5)	K D - - - - - - -	
	321 (2/2)	K D - - - - - - -	

EDI: Estimated Date of Infection. AA: amino-acid.

[a] Numbers in parentheses indicate numbers of viral sequence haplotypes with reported mutations and total number of analyzed sequences haplotypes at each time point.

investigation is necessary to determine what this host factor is, and if it is related to the HLA haplotype concordance we observed between the source and Recipient #1 (Prince et al., 2012; Gianella et al., 2011).

Methods

The source and recipient partners were identified in the San Diego Primary Infection Cohort (Hecht et al., 2010; Lundegaard et al., 2008). The estimated date of infection (EDI) of each subject was determined as previously described (Lundegaard et al., 2008). HLA typing was performed by sequence-specific PCR according to standard procedures (Gianella et al., 2012). Blood and semen were collected from each subject at baseline and HIV RNA was quantified in each fluid specimen (Beerenwinkel et al., 2003). For next generation sequencing (NGS), HIV RNA was extracted using the QIAmp kit (Qiagen, Carlsbad, CA) from blood and seminal plasma and PCR-amplified HIV-1 *env* C2-V3 (HXB2 coordinates 6928–7344), *pol* reverse transcriptase (RT, HXB2 2708-3242), and *gag* p24 (HXB2 1366-1619) were sequenced (454 FLX Roche) (Hecht et al., 2010). All NGS data were screened for in-house cross-contamination (Goulder et al., 2001), and underwent haplotype reconstruction with ShoRAH (Dalmau et al., 2009). NGS haplotypes were analyzed for co-receptor prediction with the Geno2pheno tool (Butler et al., 2010) with a false-positive rate of 5%, and underwent phylogenetic analysis using the Bayesian Markov chain Monte Carlo (BMCMC) inference implemented in BEAST v1.7.4 (Gao et al., 2001) with optimized parameters and priors (Fig. 2). Sequences were investigated for evidence of CTL escape using computationally predicted changes in MHC binding scores (O'Brien et al., 2001). Epitopes were defined as sites with a predicted IC_{50} (nM) of <100 for a HLA Class 1 molecule. Significant changes in binding affinity were defined by a greater than two fold increase in the IC_{50} (nM) using an established algorithm (O'Brien et al., 2001).

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