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Env length and N-linked glycosylation following transmission of Human Immunodeficiency Virus Type 1 subtype B viruses

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Abstract

Whether there is selection for specific viral Env variants upon HIV-1 transmission is controversial. We examined the V1V2 and V1V4 regions of Env in 10 new and 8 previously described transmission pairs infected with HIV-1 subtype B, including a total of 9 pairs in which the infecting partner had developed substantial viral diversity prior to transmission. We found that during transmission of HIV-1 subtype B, as well as for other subtypes reported in the past, viral populations in recipients undergo substantial genetic bottlenecks, as well as weak evidence for a propensity to replicate viruses with shorter variable loops and fewer potential N-linked glycosylation sites.

Keywords

HIV-1; Env sequence length; N-linked glycosylation sites; transmission; primary infection

Introduction

Understanding the properties of viruses capable of establishing infection during transmission of HIV-1 is important for vaccine design and the prevention of AIDS. In a study of 8 heterosexual transmission pairs infected with HIV-1 subtype C, Derdeyn *et al.* reported selection for viruses upon transmission with shorter length and fewer N-linked glycosylation sites predicted within the gene segment encoding the V1V4 variable regions of the Env gp120 protein (Derdeyn et al., 2004). These authors suggested that longer V1V4 and more extensive glycosylation in this region might serve to protect viruses from neutralizing antibodies. They reasoned that upon transmission into an immunologically naïve host, the shield provided by

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glycosylation was not initially required, and that such variants would be outgrown by strains with a more compact V1V4 region. A second study examined gp120 V1V2 sequences from early infection in 35 individuals heterosexually infected with HIV-1 subtype A (Chohan et al., 2005). The corresponding viral segments in these individuals were found to be significantly shorter and less glycosylated than other subtype A sequences found in the Los Alamos HIV Sequence Database (HIVDB), if database sequences derived within one year of infection were excluded. This led the authors to suggest that there was selection for viral variants with shorter V1V2 loops following transmission of HIV-1 subtype A. However, they did not find shorter or less glycosylated subtype B sequences from early infection (even using early sequences from heterosexual transmissions from the HIVDB) when compared to those from the HIVDB, again excluding subtype B sequences derived within one year of infection. In a third study involving eight subtype B infected male to male transmission pairs, no selection for shorter or less glycosylated V1V4 loops was observed in recipients (Frost et al., 2005). Of note, however, at least four of the eight infecting partners in this study were themselves newly infected at the time of transmission, and they had no evident variation in V1V4 sequence length. Reliance on rapid transmission pairs could have accounted for the failure to detect selection based on V1V4 length in the latter study. To more extensively test whether selection for virus with shorter and less glycosylated variable loops occurs following transmission of subtype B HIV-1, we studied an additional ten subtype B transmission pairs (four were identified previously (Truong et al., 2002)), including one female-to-male and nine male-to-male transmissions. The infecting partners in seven pairs had developed substantial viral diversity prior to transmission.

Results and Discussion

We obtained full or partial-length HIV-1 subtype B gp120 sequences in ten self-reported transmission pairs (Table 1). Recipients were sampled at a single time point between 0 and 199 days after onset of symptoms of primary infection, while infecting partners were sampled between 41 days before to 206 days after onset of symptoms of primary infection in the recipients. To be comparable to the results of Derdeyn *et al.* (Derdeyn et al., 2004) and Frost *et al.* (Frost et al., 2005), we performed our analysis on the V1V4 region of *env*. Consistent with reported contact histories, phylogenetic analysis of V1V4 nucleotide sequences (Figure 1) showed that for each transmission pair, putative infecting partner and recipient sequences were closely related, and formed a monophyletic cluster with respect to random sequences taken from the HIVDB. As with prior reports (Delwart et al., 1994;van't Wout et al., 1994;Wolfs et al., 1992;Zhu et al., 1993) of viral populations early in infection, we generally found a large reduction of sequence diversity in the recipients (Figure 1, Table 1). However, in three transmission pairs (Pairs A, B and C), sequences from both individuals were intermingled (Figure 1) and sequence diversities in the putative infecting partners and recipients were similar and quite low (Table 1). For pairs A and B, the dates of onset of symptoms of primary infection between the putative infecting partner and recipient are only 13 and 11 days apart; for pair C, the dates of onset of symptoms of primary infection or seroconversion of the putative infecting partner are unknown and there was no clinical evidence indicating that he was infected earlier than the recipient. Therefore, we could not assign the directionality of transmission and the three pairs were omitted from subsequent analyses.

We combined the seven pairs (Pairs D to J) above with the eight pairs from the study by Frost *et al.* (Table 2) (Frost et al., 2005). As described in Derdeyn *et al.* (Derdeyn et al., 2004), median values of sequence length and number of potential N-linked glycosylation sites (PNLGS) were calculated in each infecting partner, and the number of sequences below, equal to, or above the medians was determined. Compared to the median V1V4 sequence length within infecting partners, we found that lengths of the dominant viruses (the most common genotype) in the recipients were smaller in seven pairs, identical in five pairs, and greater in three pairs. Compared to the median number of PNLGS over V1V4 in infecting partners, the dominant

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viruses in the recipients had fewer in eight pairs, identical numbers in four pairs, and more in three pairs (Table 2). In nine pairs, the V1V4 length and glycosylation levels were both concordant. To assess the likelihood of this distribution, we performed the non-parametric statistical test described in Derdeyn *et al.* (perl script kindly provided by B. Korber) (Derdeyn et al., 2004). Assuming that all infecting partner sequences were equally likely to be transmitted and grow out in the recipient, and given the distribution of the sequence length and number of PNLGS in V1V4 in the infecting partners, the probabilities of observing virus in seven or more recipients with shorter V1V4 lengths, and eight or more with a lower number of PNLGS in fifteen pairs were $p = 0.021$ and $p = 0.003$, respectively. This result indicates a selection for shorter and less glycosylated V1V4 loops. However, as shown below, a total of 8 tests were performed, and the statistical significance we reported only remained for the PNLGS result (for V1V2 and V1V4 regions) after Bonferroni correction for multiple tests.

Chohan *et al.* reported that sequences encompassing the V1V2 region of Env from early HIV-1 subtype A infections were significantly shorter and had fewer PNLGS than the overall population of circulating viruses (Chohan et al., 2005). We therefore performed an analysis of our transmission pairs confined to the V1V2 region. Dominant V1V2 lengths in recipients were shorter than, identical to and longer than the infecting partner medians in five, nine and one pair(s), respectively. The numbers of PNLGS in dominant forms of virus in recipients were smaller than, identical to and greater than the infecting partner medians in six, seven, and two pairs, respectively. The probabilities of observing five or more recipients with shorter V1V2 sequences, and observing six or more recipients with fewer PNLGS in fifteen pairs, were $p = 0.086$ and $p = 0.0003$, respectively. For the three pairs (Pairs A to C) with uncertain transmission directionality, the sequence lengths and numbers of PNLGS in V1V2 and V1V4 were identical between the putative infecting partners and recipients in two cases, and greater in the recipient in one.

We reasoned that detection of selection was likely to be facilitated by high viral genetic diversity in infecting partners. The highest diversity among all putative recipients was 0.66% (Table 1), whereas viral diversities in the infecting partners of nine pairs in our combined data set were over 0.66%, ranging from 1.47–6.11%. We therefore performed a sub-analysis on these nine pairs (Table 2, the groups of 7 in upper panel, and 2 in lower panel, below the dotted lines). All these infecting partners had variance in sequence length and number of PNLGS. Surprisingly, we did not find selection for viral population with shorter or less glycosylated V1V2 or V1V4 loops in the recipients (all p values >0.1; data not shown).

In summary, our study reaffirmed the finding that substantial genotypic bottlenecks occur during and/or closely following transmission (Delwart et al., 1994;van't Wout et al., 1994; Wolfs et al., 1992;Zhu et al., 1993). We have also shown for the first time in HIV-1 subtype B, selection for viral variants with shorter variable regions and a reduced degree of glycosylation following transmission to a new host. However, this demonstration was dependent upon how pairs were chosen for inclusion. It should be stressed that we expected that this difference would be greater when choosing only infecting partners with substantial viral diversity prior to transmission, and this was not the case. Furthermore, no study so far has found these reductions to be consistent within all or a large majority of recent HIV-1 infections (e.g., shorter loop lengths and fewer PNLGS were found in 6/8 and 5/8 cases, respectively, reported by Derdyen (Derdeyn et al., 2004), and 7/15 and 8/15 cases, respectively, for V1V4 in this study). In addition, the distributions of both lengths and PNLGS numbers overlapped substantially between the two groups representing early and chronic infections, in the Chohan study (Chohan et al., 2005).

A high proportion (nearly one-half) of transmissions are thought to occur when the infecting partner is in the early stages of infection (Pilcher et al., 2004; Wawer et al., 2005). This is

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certainly a component of why the issue of selection in these regions of Env remain unsettled. The statistically significant selection we found for HIV-1 Subtype B infections occurred as sample sizes of pairs with substantial diversity in infecting partners have grown - all eight of the infecting partners evaluated by Derdeyn had high levels of diversity. The basis for this selection is thought to represent a trade-off between the advantages of antibody neutralization escape, afforded by addition of PNLGS, and replication fitness in a naïve immune environment (Derdeyn et al., 2004). The same phenomenon has been detected for SIV grown ex vivo in cell culture versus passaged in macaques (Edmonson et al., 1998). The failure to routinely detect this selection suggests that the fitness loss associated with neutralization escape is not always substantial.

Materials and Methods

Sequence generation and analysis

Plasma viral sequences were obtained using nested PCR as previously described (Liu et al., 2006). For *gp120*, the first round forward primer was ED3 (Delwart et al., 1995) (HIV-1_{HXB2} 5957–5986), and the reverse primer was nef3 (Shankarappa et al., 1999) (HIV-1_{HXB2} 9038-9015). The second round forward primer was gp120-5: GGCCGCGTCGACAAGAGCAGAAGACAGTGGCAATGA (HIV-1_{HXB2} 6194–6228), and the reverse primer was gp120-3: GGCCGCGGATCCGTGCTTCCTGCTGCTCCCAAGAAC (HIV-1_{HXB2} 7823 - 7787). For V1–V5, the first round forward primer was ED3 and the reverse primer was ED14 (Delwart et al., 1993) (HIV-1_{HXB2} 7961-7932). The second round forward primer was ED5 (Delwart et al., 1995) (HIV-1_{HXB2} 6557-6577), and the reverse primer was ED12 (Delwart et al., 1993) (HIV-1_{HXB2} 7811-7791). PCR products were cloned into TOPO vector (Invitrogen, San Diego, CA) and selected for sequencing as described previously (Shankarappa et al., 1999) to avoid template resampling (Liu et al., 1996). Sequences were determined with an automated DNA sequencer (Applied Biosystems, Foster City, CA) and edited using SEQUENCHER, version 3.0 (Gene Codes Corp., Ann Arbor, MI). Genbank access numbers will be provided. V1V4 nucleotide sequences from each subject were aligned using the program MacClade (Maddison and Maddison, 2001) with 114 randomly chosen HIV-1-B sequences from the Los Alamos Sequence Database (one sequence per individual). Neighbor-joining tree was constructed using HKY85 model with the program PAUP* (Swofford, 1999), and average pair-wise distance was calculated. Numbers of PNLGS were calculated using tool N-Glycosite (Zhang et al., 2004) from HIVDB.

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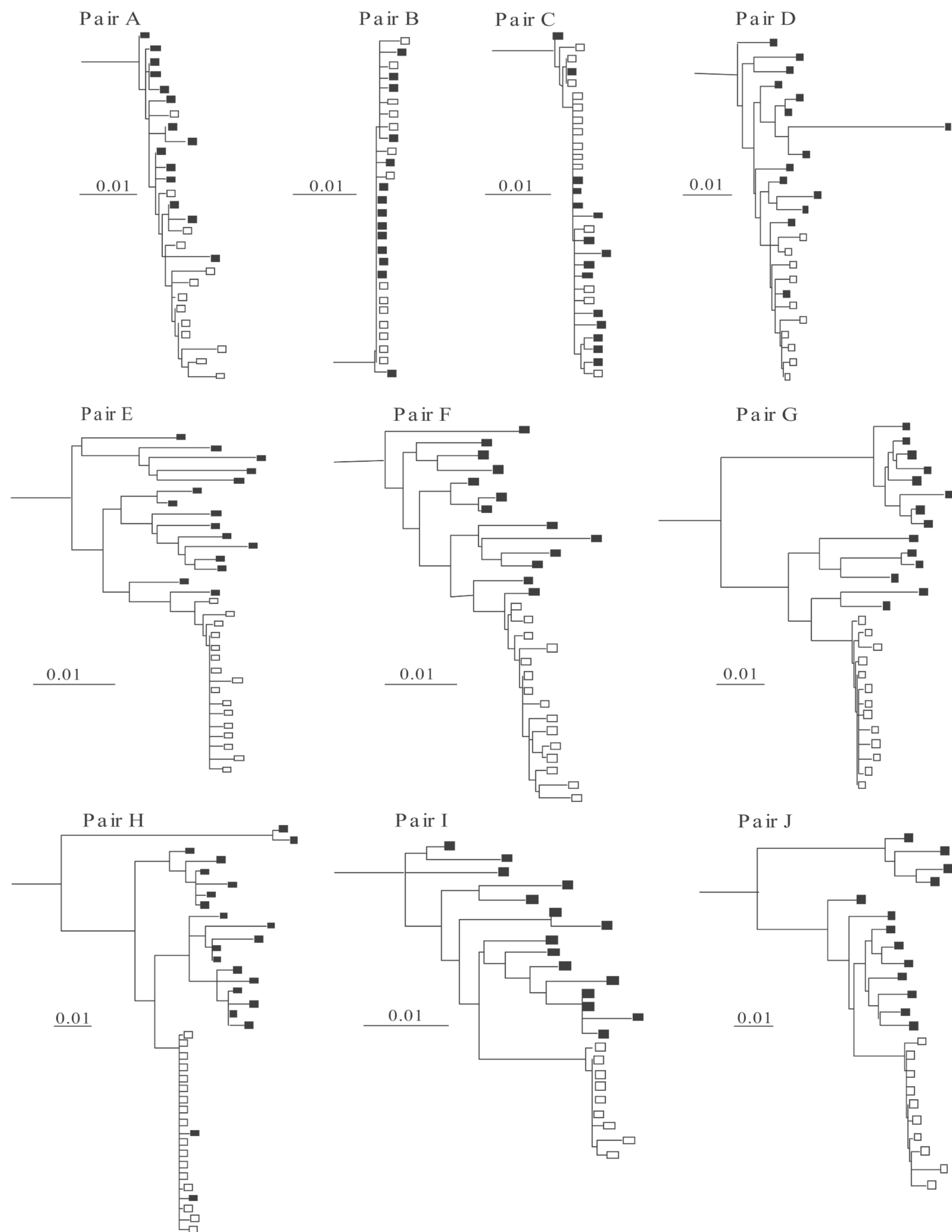

Figure 1. Rooted neighbor-joint trees of the putative infecting partner-recipient pairs

To assess possible infecting partner-recipient relationships, V1V4 nucleotide sequences from each subject were aligned with 114 randomly chosen HIV-1-B sequences from the Los Alamos Sequence Database (one sequence per individual). Neighbor-joining trees were rooted with the 114 sequences from unrelated individuals (not shown in the trees). Filled rectangles represent sequences from reported infecting partners and empty rectangles represent those from reported recipients, which in each case formed a monophyletic cluster distinct from all reference sequences.

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Table 1

Donors and recipients in HIV-1 subtype B infected transmission pairs

Pairs[a]		*env* region	Number of sequences		Estimated length of IP infection[b]	Sampling time (days)[c]		Pair-wise distance of V1V4 (%)[e]	
			IP[d]	R[d]		IP	R	IP	R
A		gp120	14	13	94 d	81	92	0.61	0.66
B		gp120	14	14	5 d	−6	0	0.25	0.11
C		V1V5	15	14	<2yr	206	199	0.40	0.25
D		gp120	15	10	~4 m	−41	56	2.11	0.62
E		gp120	15	16	58 d	56	51	2.07	0.23
F		gp120	15	15	2 y	49	29	2.60	0.49
G		V1V5	14	13	~20 y	151	13	6.11	0.49
H		V1V5	21	17	10 y	23	8	4.61	0.11
I		V1V5	15	9	~6 y	65	42	2.33	0.23
J		V1V5	13	10	~ 4 y	29	29	4.57	0.46
Pairs from study by Frost et al. (Frost et al., 2005)									
IP	R				IP stage of infection	Days between sampling of IP and R			
0007	0004	gp160	11	13	Recent	15		0.34	0.18
0512	0558	gp160	11	14	Recent	−94		0.15	0.26
0512	0559	gp160	11	11	Recent	−94		0.15	0.21
0551	0550	gp160	13	12	Recent	0		0.31	0.32
0206	0201	gp160	13	11	Chronic	16		0.62	0.17
0206	0204	gp160	13	12	Chronic	3		0.62	0.32
0465	0449	gp160	11	12	Chronic	32		2.78	0.21
0564	0557	gp160	14	15	Chronic	14		1.47	0.34

[a] Four of the pairs have been reported previously (Truong et al., 2002). Pairs C, H, I and J in this study correspond to pairs G, E, K and B in the previous study, respectively. Pair J here is a heterosexual pair, with a female donor. In addition, additional chart reviews for pair C in this study revealed that the directionality of transmission reported previously (Truong et al., 2002) as well as the dates of infection reported in that paper were most likely in error. We were not able to determine the directionality of transmission for this pair using genetic analysis and clinical information.

[b] Days (d), months (m) or years (y) between the onset of acute symptoms of primary infection or first positive antibody test and sampling in the donor.

[c] Days between the onset of acute symptoms of primary infection in the recipient and sampling of donor or recipient.

[d] IP, putative infecting partner; R, putative recipient.

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Table 2

Comparison of V1V4 and V1V2 in donors and recipients in HIV-1 subtype B infected transmission pairs.

Pairs		Amino acid length of V1V4		PNLGS[a] in V1V4		Amino acid length of V1V2		PNLGS in V1V2	
		IP[b] Median (range)[c]	R[b] Dominant (range)	IP Median (range)	R Dominant (range)	IP Median (range)	R Dominant (range)	IP Median (range)	R Dominant (range)
A		285(279–286)	285(278–285)	20(18–21)	20(19–20)	67(61–67)	67(60–67)	4(3–5)	4(3–5)
B		282(282–288)	282	20(20–21)	20	66(66–72)	66	5	5
C		290(290–293)	293[d](297–293)	21(20–22)	22(21–22)	72(72–75)	75(61–75)	4(3–5)	5
D		278(275–282)	278(269–278)	21(18–22)	21(19–21)	58(55–62)	58	4(3–5)	4
E		297(283–313)	297	24(22–27)	23(22–23)	81(67–97)	81	8(7–11)	8(7–8)
F		287(243–296)	283(230–283)	24(18–26)	21(17–21)	69(66–78)	68	7(7–9)	6(5–6)
G		307(295–317)	291(291–292)	24(20–27)	21(20–21)	91(79–99)	75(75–76)	10(6–12)	6
H		290(289–302)	292	23(22–24)	22(21–22)	70(69–81)	70	7(6–8)	7(6–7)
I		286(279–288)	288	21(19–22)	23(22–23)	69(67–69)	69	7(6–7)	8
J		287(283–289)	287	21(20–22)	22	68(67–68)	68	6	6
Pairs from study by Frost et al.(Frost et al., 2005)									
IP	R								
0007	0004	294	294	23(22–23)	22(20–22)22(20–22)		77	7	6
0512	0558	295	288	25(24–25)	22(21–22)	73	66	7	4
0512	0559	295	288	25(24–25)	22(21–22)	73	66	7	4(3–4)
0551	0550	288	288	22	22(20–22)	72	72	6	6
0206	0201	283(281–283)	281	20(20–22)	20	63	63	5(5–6)	5
0206	0204	283(281–283)	281	20(20–22)	20(19–20)	63	63	5(5–6)	5(4–5)
0465	0449	286(280–289)	295	21(19–21)	22(21–22)	70(64–73)	79	6(4–7)	8
0564	0557	301(299–303)	291	25(23–26)	21(19–21)	82(81–82)	72	9(7–9)	6(5–6)

[a] Potential PNLGS.

[b] IP, putative infecting partner; R, putative recipient.

[c] Range shown if population was not monotypic.

[d] Larger median or dominant numbers of the pairs are highlighted.