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Phenotypic properties of envelope glycoproteins of transmitted HIV-1 variants from patients belonging to transmission chains

Short title: Env properties of transmitted viruses

Original paper

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ABSTRACT

Objective: Transmission of HIV-1 involves a bottleneck in which generally a single HIV-1 variant from a diverse viral population in the transmitting partner establishes infection in the new host. It is still unclear to what extent this event is driven by specific properties of the transmitted viruses or the result of a stochastic process. Our study aimed to better characterize this phenomenon and define properties shared by transmitted viruses.

Design: We compared antigenic and functional properties of envelope glycoproteins of viral variants found during primary infection in 27 patients belonging to eight transmission chains.

Methods: We generated pseudotyped viruses expressing Env variants of the viral quasi-species infecting each patient and compared their sensitivity to neutralization by eight human monoclonal broadly neutralizing antibodies (HuMoNAbs). We also compared their infectious properties by measuring their infectivity and sensitivity to various entry inhibitors.

Results: Transmitted viruses from the same transmission chain shared many properties, including similar neutralization profiles, sensitivity to inhibitors, and infectivity, providing evidence that the transmission bottleneck is mainly non-stochastic. Transmitted viruses were CCR5-tropic, sensitive to MVC, and resistant to soluble forms of CD4, irrespective of the cluster to which they belonged. They were also sensitive to HuMoNAbs that target V3, the CD4 binding site, and the MPER region, suggesting that the loss of these epitopes may compromise their capacity to be transmitted.

Conclusions: Our data suggest that the transmission bottleneck is governed by selective forces. How these forces confer an advantage to the transmitted virus has yet to be determined.

Keywords: HIV transmission, bottleneck, selection, envelope glycoproteins properties

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INTRODUCTION

The HIV-1 population found in acute infections is very homogeneous, in contrast to the high diversity of the quasispecies observed in chronic infections [1,2]. Several studies concluded that sexual transmission of HIV-1 involves a genetic bottleneck, resulting in the transmission of a single or limited number of viral variants [2–8].

Substantial efforts have been made to identify properties that differ between transmitted/founder (T/F) and chronic viruses. However, many findings are inconsistent. T/F viruses from subtypes A, C, and D appear to carry shorter and less glycosylated envelope glycoproteins (Env) [1,9,10], but this trend was not observed in subtype B viruses [5,11,12]. Clade B T/F viruses were reported to be more sensitive to neutralizing antibodies (NAbs) that target the CD4 binding site [12], but this was not observed for subtype C viruses [10]. On the other hand, subtype C T/F viruses were found to be more sensitive to antibodies obtained from their infecting donor [1], but this was not observed in a subtype B cohort [11]. Several studies have shown that almost all T/F viruses use the CCR5 co-receptor and require high levels of CD4 to infect cells [3,10,13–16], even if T/F viruses appear to be more restricted in the use of various CCR5 conformations than chronic viruses [10,17]. Recent papers suggested that dendritic cells or immature Langerhans cells mediate the selective transmission of CCR5-using viruses during their penetration through the mucous membrane barriers [18,19]. T/F viruses appear also to be modestly more resistant to the fusion inhibitor T1249 than chronic viruses [3], but this was not observed using the fusion inhibitor enfuvirtide (T20) [20]. T/F viruses were reported to display enhanced binding to dendritic cells and to subsequently be more efficiently transferred to T cells than viruses derived from chronic infections [21], but this was not observed in another study [12]. It was also shown that T/F viruses exhibit enhanced infectious properties [21,22], but this trend was not observed in other studies [5,23]. Finally, it was suggested that the innate immune system, through type I interferons, may favor

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the selection of interferon α (IFNα)-resistant viruses during transmission [21,22,24,25]. In contrast, other studies showed that T/F viruses were equally [23] or modestly more [5] sensitive to IFN-α than their linked donor isolates.

Overall, except for CCR5 utilization, no phenotypic features are consistently linked to the transmitted variant. Conflicting findings may originate from differences in methodologies and/or study populations. Indeed, some studies examined only a limited number of transmission pairs using samples issued from the chronically infected donor and its linked receiver, while others used large sequence data sets of unlinked acutely- versus chronically infected patients. However, we cannot exclude that stochastic effects may also contribute to the transmission bottleneck and that any sufficiently fit R5 virus might be transmitted.

To better understand whether the transmission bottleneck is mainly stochastic or if viral variants with particular biological properties are selected at transmission, we used another approach based on the comparison of the antigenic and functional properties of transmitted viruses that have a common origin, derived from eight transmission clusters. We hypothesized that, if the transmitted variants within a transmission cluster have a selective advantage, they should share some phenotypic characteristics. Moreover, if some properties are conserved between all the variants irrespective of the cluster to which they belong, they should favor their transmission.

METHODS

Study population

The HIV-1 population was derived from plasma samples collected at time of or very early after primary infection from patients enrolled in the ANRS PRIMO cohort [26]. The patients were 27 Caucasian men having sex with men (MSM), infected by clade B (six clusters, 21 patients) or CRF02-AG (two clusters, six patients) viruses between 2006 and 2013 (Supplemental Table 1, http://links.lww.com/QAD/B314). They segregated into eight

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transmission clusters identified by strong similarity of the *pol* gene (genetic distance < 0.015%) and phylogenetic analyses (bootstrap of the cluster > 98%) [27]. All patients were antiretroviral-naive at the time of enrollment in the cohort. The estimated date of infection was defined as the date of symptom onset minus 15 days for patients with symptomatic primary infection, or, for asymptomatic patients, the date of the incomplete western blot (presence of antibodies to gp160 and P24) minus 1 month or the midpoint between a negative and a positive ELISA result [26]. National ethics committee approvals were obtained for the cohort [Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale (CCPPRB) Paris-Cochin and Comité de Protection des Personnes (CPP) Ile de France III] and all patients gave written informed consent to participate in the cohort.

Production of pseudotyped viruses, titration, and analysis of their Env content

Env-pseudotyped viruses were produced as described previously [28]. For the analysis of the Env content, viral particles were overlaid on a 20% sucrose cushion and pelleted at 87,000 x *g* for 1.5 h at 4°C. Viral pellets were solubilized overnight at 4°C in 100 µl PBS supplemented with 1% Triton X-100 and an antiprotease cocktail. P24 antigen content was determined by ELISA (INNOTEST® HIV Antigen mAb; Innogenetics). The Env ELISA was performed in Nunc Maxisorp plates (Dutscher). A pool of three HuMoNAbs (PGT145, b12, PGT128) was used for the detection of Env captured on D7324 (Aalto Bioreagents Ltd., Dublin, Ireland)-coated microplates. Dilutions of purified gp120IIIB (Advanced Bioscience Laboratories) were used to construct a standard curve.

Viral infectivity in TZM-bl cells

Viral infectivity was determined in quadruplicate in TZM-bl cells. Samples of 100 µL virus stock, normalized to 25 ng P24 were added to 100 µL culture medium. Aliquots of 1 x 10^4 TZM-bl cells were added to viruses in the presence of 30 µg/mL DEAE-dextran. Infection levels were determined after 48 h by measuring the luciferase activity of cell lysates using the

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Bright-Glo luciferase assay (Promega) and a Centro LB 960 luminometer (Berthold Technologies).

Neutralization assay

The sensitivity to neutralization of pseudotyped viruses was probed in TZM-bl cells using HuMoNAbs PG9, PGT145, NIH45-46^{G54W}, 3BNC117, 10-1074, PGT121, 10E8 and 8ANC195 (IAVI and NIH AIDS Reagent Program), as described previously [28].

Determination of co-receptor usage

Co-receptor usage was determined using U373 MAGI cells (NIH AIDS Reagent Program) stably expressing CD4 and either CCR5 or CXCR4, as described previously [29].

Inhibition of entry by Enfuvitide, CCR5 antagonists, and CD4 analogs.

TZM-bl cells were used in duplicate to assess the sensitivity of pseudotyped viruses to the fusion inhibitor enfuvirtide (T20), CCR5 antagonist MVC, and CD4 inhibitors sCD4, sCD4-183 (NIH AIDS Reagent Program), and M48U1 (provided by L. Martin, CEA, Gif sur Yvette, France). After titration, pseudotyped virus stocks were diluted to obtain 400 $TCID_{50}$/mL in growth medium. Aliquots of 50 µL were then incubated for 1 h at 37°C with 50 µL of three-fold serial dilutions of T20, sCD4, sCD4-183 or M48U1 (10 µg/mL to 0.0046 µg/mL). The virus-inhibitor mixture was then used to infect 1 x 10^4 TZM-bl cells in the presence of 30 µg/mL DEAE-dextran. Infection levels were determined after 48 h by measuring the luciferase activities of cell lysates. IC_{50} values were defined as the inhibitor concentration reducing RLUs by 50%. Results were expressed as mean duplicate values.

For MVC inhibition, 8 x 10^3 TZM-bl cells per well were plated the day before infection. Cells were first treated for 1 h at 37°C with 150 µL three-fold serial dilutions of MVC (6 µM to 0.3 nM) before adding 50 µL pseudotyped viruses normalized to 400 $TCID_{50}$/mL. One hundred microliters DMEM medium, supplemented with 30 µg/mL DEAE-dextran, was then added to

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the cells. Luciferase activity was measured 48 h after infection as described above. CCR5 antagonist susceptibility is expressed as the maximal percent inhibition (MPI) and IC_{50} values.

Sanger Sequence analysis

All full-length *env* PCR products were sequenced according to the Dye Terminator cycle sequencing protocol (Applied Biosystems, Foster City, Calif.). All sequences have been submitted to GenBank and assigned accession numbers MH000288 to MH000314. Nucleotide sequences were aligned using CLUSTALW and manually edited. Potential N-linked glycosylation sites (PNGS) were identified using the N-Glycosite tool at the HIV LANL database website (http://www.hiv.lanl.gov). A maximum likelihood tree was computed with RAxML, using the GTRGAMMA model of nucleotide substitutions with bootstrap analysis to assess branch support (1,000 replicates). We conducted a BLAST search to identify the 10 most closely related sequences available in Genbank for each sequence. After excluding duplicate sequences, these sequences were downloaded and included in the phylogenetic analysis. Mean intra-cluster p-distances, i.e. the proportion of nucleotide differences between two sequences in a cluster, were calculated using MEGA7.

RESULTS

Studied population.

Blood samples of selected patients were collected less than three months post-infection. The variants that we analyzed were therefore considered to be early/transmitted viruses. The maximum window periods for transmission after primary infection were estimated by determining the time interval between the first and last infection within each cluster (Supplemental Table 1, http://links.lww.com/QAD/B314). Transmission intervals ranged from 0.4 to 37.6 months, with a median transmission interval of 17.1 months (IQR 5–35.5).

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Comparison of nucleotidic *env* and deduced amino-acid Env sequences

Full length *env* sequences of the transmitted viral population infecting each subject were obtained by sequencing of bulk *env* PCR products. We compared the 27 sequences to the 140 most closely related *env* sequences available in Genbank. Phylogenetic analyses confirmed that the *env* sequences from the selected patients grouped into eight clusters (bootstrap value of 100%), including six clusters of subtype B and two clusters of CRF02-AG *env* sequences. Within each cluster, the mean p-distance was low, ranging from 0.005 to 0.093 nucleotide substitutions per site (Figure 1).

Because it was previously suggested that short length and low number of PNGS of gp120 variable regions were characteristics of T/F viruses [1,9,10], we looked at them in our 27 Env sequences (Table 1). Both length and number of PNGS differed between clusters. Surprisingly, although variants within each cluster were genetically closely related, a substantial diversity in length and number of PNGS of the variable regions was observed, except for the V3 region. Only variants of cluster 8 were highly homogeneous, with equal length, net charge, and PNGS for each variable region. Of note, the time interval between the first and last infection within this cluster was extremely short (11 days) (Supplemental Table 1, http://links.lww.com/QAD/B314).

Transmitted viruses belonging to the same cluster have similar neutralization profiles

We generated pseudotyped viruses expressing Env variants representative of the viral quasi-species infecting each patient and compared their sensitivity to neutralization by a panel of eight HuMoNAbs chosen to be representative of the five regions of vulnerability of the HIV-1 envelope [30–44] (Table 2). Neutralization profiles were highly conserved within each cluster except for viruses of cluster 6, suggesting the transmission of variants with specific properties, even when the transmission events occurred at several months interval. Viruses of cluster 6 differed from those of other clusters by their high resistance to almost all HuMoNAbs.

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Disregarding variants of cluster 6, all transmitted viruses were susceptible to antibodies targeting V3, the CD4 binding site, and the MPER region, suggesting the transmission of viruses harboring these epitopes. The neutralization profiles, although similar within each cluster, were different between clusters. This observation suggests a conservation of specific properties within each cluster for the viruses to be transmitted, but conservations that can be different for each transmission chain.

Inspection of HuMoNAb epitopes on Env sequences showed that most resistant viruses harbored mutations of essential residues (Supplemental Fig. 1, http://links.lww.com/QAD/B314). We observed a shift of the PNGS from N332 to N334 in all viruses of cluster 6 resistant to antibodies targeting V3 (Supplemental Fig. 1A, http://links.lww.com/QAD/B314), a shift of the PNGS from N234 to N230 in viruses of clusters 3, 4, and 6, resistant to 8ANC195 (Supplemental Fig. 1B, http://links.lww.com/QAD/B314), and the loss of N160 in viruses of clusters 6 and 7 that were resistant to both PG9 and PGT145 (Supplemental Fig. 1C, http://links.lww.com/QAD/B314). We did not find any potential relationship between the resistance of viruses to antibodies targeting the CD4 binding site and MPER region and the absence of specific residues at key positions.

Transmitted viruses exhibit CCR5 tropism and are highly susceptible to maraviroc (MVC)

We first determined the tropism of pseudotyped viruses by measuring their ability to efficiently infect $CD4^{+}$/U373 MAGI cells expressing either the CXCR4 or CCR5 co-receptor [45]. All viruses infected exclusively $CD4^{+}$/$CCR5^{+}$/U373 cells, as expected (Supplemental Table 2, http://links.lww.com/QAD/B314).

We then assessed the efficiency of CCR5 usage of Env-pseudotypes by infecting TZM-bl cells in the presence of decreasing concentrations of the CCR5 antagonist MVC (6000 nM to

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0.3 nM). All pseudotyped viruses were highly susceptible to MVC with an IC_{50} < 10 nM and maximal percent inhibition (MPI) values above 90% in the presence of saturating concentrations of MVC (Table 3).

Transmitted viruses are resistant to soluble CD4 but sensitive to a CD4-mimetic miniprotein

We compared the efficiency of CD4 receptor usage of all pseudotyped viruses through their sensitivity to CD4 analogs. Most viruses were highly resistant to two soluble forms of CD4 containing either all four (sCD4) or the first two (sCD4-183) extracellular domains, with IC_{50} values > 10 µg/mL for 21 and 23 of 27 viruses, respectively (Table 3). In contrast, all pseudotyped viruses, except one variant of cluster 6, were sensitive to M48U1, a CD4 mimetic miniprotein [46,47] (Table 3).

We compared residues of the Phe-43 cavity and those known to interact with CD4 and/or M48U1 in the 27 Env sequences to identify potential molecular determinants of these differences (supplementary Figure 2, http://links.lww.com/QAD/B314) [32,46–49]. There were no significant differences between viruses resistant or sensitive to the two soluble forms of CD4. Comparison of the residues that interact with M48U1 showed that the only resistant virus (virus 275 of cluster 6) harbored a mutation of the conserved serine/threonine residue at position 375 to an isoleucine residue. The loss of this residue has already been described to disrupt the molecular interaction with M48U1 [47,50,51].

Transmitted viruses are sensitive to the fusion inhibitor enfuvirtide (T20)

We tested Env-pseudotyped viruses for their sensitivity to T20. By mimicking the activity of HR2, T20 binds to the HR1 domain of gp41, which becomes exposed after CD4/coreceptor engagement. Higher resistance to this fusion inhibitor may thus reflect faster fusion kinetics. All viruses were sensitive to T20 (IC_{50} range: < 0.00457 to 1.23 µg/mL), with conserved sensitivity levels within each transmission cluster (Table 3).

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Transmitted viruses have similar infectious properties

We compared the capacity of pseudotyped viruses to infect TZM-bl cells in a single round of infection. Infectivity levels of each pseudotyped virus, for which the input was normalized to the amount of P24, was evaluated 48 h post-infection by measuring luciferase activity (RLU). Infectivity levels were conserved within clusters, with a relative standard deviation (RSD) of $\log_{10}$ RLU < 15% for almost all clusters (Table 4). Only two clusters (clusters 2 and 6) displayed higher variability of infectivity levels (RSD of $\log_{10}$ RLU > 15%). Infectivity levels were also conserved between clusters, with $\log_{10}$ RLU means ranging from 5.59 to 6.65 and a global RSD of 10.71% for the 27 viruses. Since Env incorporation may be variable among the pseudotyped viruses, we determined the gp120 content of each viral stock and we assessed the infectivity after normalization for Env content. The four viruses of cluster 6 and one virus (virus 256) of cluster 2 harbored very low levels of gp120, under the detection limit of the ELISA test (Table 4). Except for these five viruses, we evaluated the infectivity of each pseudotyped virus normalized for Env content by dividing infectivity values by gp120 content. Infectivity levels were again conserved, both within (RSD of $\log_{10}$ RLU < 10%) and between clusters (global RSD of 7.39 % for the 22 viruses for which the infectivity values could be normalized) (Table 4). Overall, these results show that transmitted viruses had similar infectious properties.

DISCUSSION

Previous studies have shown that generally a single HIV-1 variant from the diverse viral population in the transmitting partner establishes infection in the new host [2,3,5]. HIV-1 is clearly subjected to a stringent genetic bottleneck during transmission, but the extent to which this event is driven by specific properties of the transmitted viruses or is the result of a stochastic process remains to be determined. We analyzed the antigenic and functional properties of envelope glycoproteins of viral variants present during or very early after

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primary infection in 27 patients belonging to eight transmission chains of at least three patients to better understand this phenomenon. Our strategy was based on the assumption that, if transmitted viruses share properties within a transmission chain, these would be indicative of a non-stochastic process and, if properties are conserved both within and between transmission clusters, these would reflect a selective advantage.

Sequencing and phylogenetic analysis of *env* genes confirmed the close relation between viruses from a same transmission cluster. A detailed comparison of deduced Env amino-acid sequences revealed that the V1, V2, V4 and V5 regions differed in length, number of PNGS, and charge, despite the fact that the sequences were closely related within each cluster. Thus, transmitted variants, albeit closely related, differed between patients of the same transmission chain. Although we could assume that the viruses belonged a same transmission chain based on molecular evidences, a comparison of transmitted variants with viral populations circulating in the chronic phase of infection could not be done because almost patients were treated within a few months after their inclusion in the cohort. In addition, we did not have epidemiological data suggesting that there were direct links such as donor/receiver pairs within them. It can be hypothesized that some transmission events might have emerged from other individuals belonging to the chain, including chronically infected patients harboring a diversified viral population. This is supported by studies on MSM cohorts from different countries that estimated that only approximately 25% of transmissions occurred during the first six months of infection [52–54].

We analyzed the antigenic profiles of the transmitted Env variants by measuring their sensitivity to a panel of representative HuMoNAbs targeting the five regions of vulnerability of the HIV-1 envelope. Although the transmitted variants were not identical, they shared similar profiles. Neutralization profiles were highly conserved within all but cluster 6, regardless of the HuMoNAb, suggesting that the transmission bottleneck is mainly driven by

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a non-stochastic process. In addition, disregarding viruses of cluster 6, all transmitted viruses were susceptible to antibodies targeting V3, the CD4 binding site, and the MPER region, strongly suggesting the transmission of viruses harboring these epitopes. These results are in accordance with our previous study showing that the best combination of HuMoNAbs to efficiently neutralize recently transmitted viruses of subtype B was that targeting these three regions [55]. These HuMoNAbs should therefore be good candidates to limit HIV transmission in prophylactic approaches or vaccine strategies. However, a variant such that present in cluster 6 would be susceptible to escape to prophylaxis with this tri-specific combination. This observation, in addition to the fact that the HIV-1 species seems to evolve toward higher resistance to neutralization [28,55-57], argues for a global prospective surveillance of the susceptibility of HIV-1 to HuMoNAbs.

We explored the functional properties of the transmitted viruses by next comparing their sensitivity to various entry inhibitors, including the MVC CCR5 antagonist, several CD4 analogs, and the T20 fusion inhibitor. All viruses were CCR5-tropic and highly susceptible to MVC, suggesting that transmitted viruses are restricted to use CCR5 conformations that are sensitive to MVC. This result is in accordance with previous studies showing that transmitted viruses are less able to use a MVC-resistant form of CCR5 than viral variants found during chronic infections [10,17]. Although the biological significance of this phenomenon remains to be determined, it appears that the transmission bottleneck selects for viruses that are restricted in the use of the CCR5 co-receptor. Analysis of the sensitivity of transmitted viruses to two soluble forms of CD4 (sCD4 and sCD4-183) showed that most viruses were highly resistant to both forms (IC_{50} > 10 µg/mL), suggesting that their affinity for CD4 is low and consequently that they may require high levels of CD4 to efficiently infect target cells. In agreement with our results, several studies have shown that transmitted viruses are unable to efficiently infect cells expressing low levels of CD4 [14,15,58,59]. In contrast, all viruses

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were highly sensitive to M48U1. The difference in response to exposure to either soluble forms of CD4 or M48U1 could be explained by the fact that M48U1 binds to gp120 with higher affinity than that of the CD4 protein [46,60]. This property is due to the presence of flexible hydrophobic extensions that fit into and stabilize the Phe-43 cavity, a critical element in the interaction between gp120 and CD4. Our result reinforces the potential use of M48U1 as a new microbicide to prevent HIV-1 transmission [61]. Viruses were also highly sensitive to the fusion inhibitor T20, with conserved sensitivity levels within each cluster reinforcing the hypothesis that the transmission bottleneck is a non-stochastic event.

We also analyzed the infectivity of transmitted viruses in a single round of infection of TZM-bl cells. Except for a few viruses, transmitted viruses shared similar infectious properties, regardless of whether the input normalization was based on P24 or Env incorporation levels. Infectivity levels were in the same range as those of regularly used laboratory strains, NL4-3 or AD8, suggesting that the transmission bottleneck did not select for particularly highly infectious Env variants.

In conclusion, our study shows that transmitted viruses from the same transmission chain share similar phenotypic properties. It is impressive to see that viruses from a same cluster have similar neutralization profiles and sensitivity to various inhibitors, even when viruses are transmitted at several months or years interval and possibly after passages through various intermediate hosts. It suggests that the conservation of biological properties for a given variant to be successfully transmitted is a necessity. It seems to us that this phenomenon is a strong argument to suggest that the transmission bottleneck is mainly non-stochastic but involves specific properties conferring a selective advantage. In addition, some properties were conserved between all viruses, regardless of the cluster they belong. They were CCR5-tropic, sensitive to MVC, and resistant to soluble forms of CD4, reinforcing previous studies showing the restrictive use of CCR5 and the requirement of high levels of CD4 of transmitted

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viruses. They were also sensitive to HuMoNAbs targeting V3, the CD4 binding site, and the MPER region, suggesting that the loss of these functionally important regions might compromise their fitness and transmission capacity. Taken together, our results argue for the necessity for a variant to harbor specific phenotypic properties to be able to be transmitted.

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FIGURE LEGENDS

Figure 1. Midpoint-rooted maximum likelihood phylogenetic tree of full-length *env* sequences. The 27 full-length *env* sequences of the transmitted viruses included in our study were aligned with the 140 most closely related sequences available in Genbank. A maximum likelihood tree was constructed using RAxML. Transmission clusters are identified by different colors. Bootstrap values (%) and the mean intra-cluster p-distances of transmission clusters are indicated (as nucleotide substitutions per site). Branch lengths correspond to nucleotide substitutions per site, as indicated in the scale.

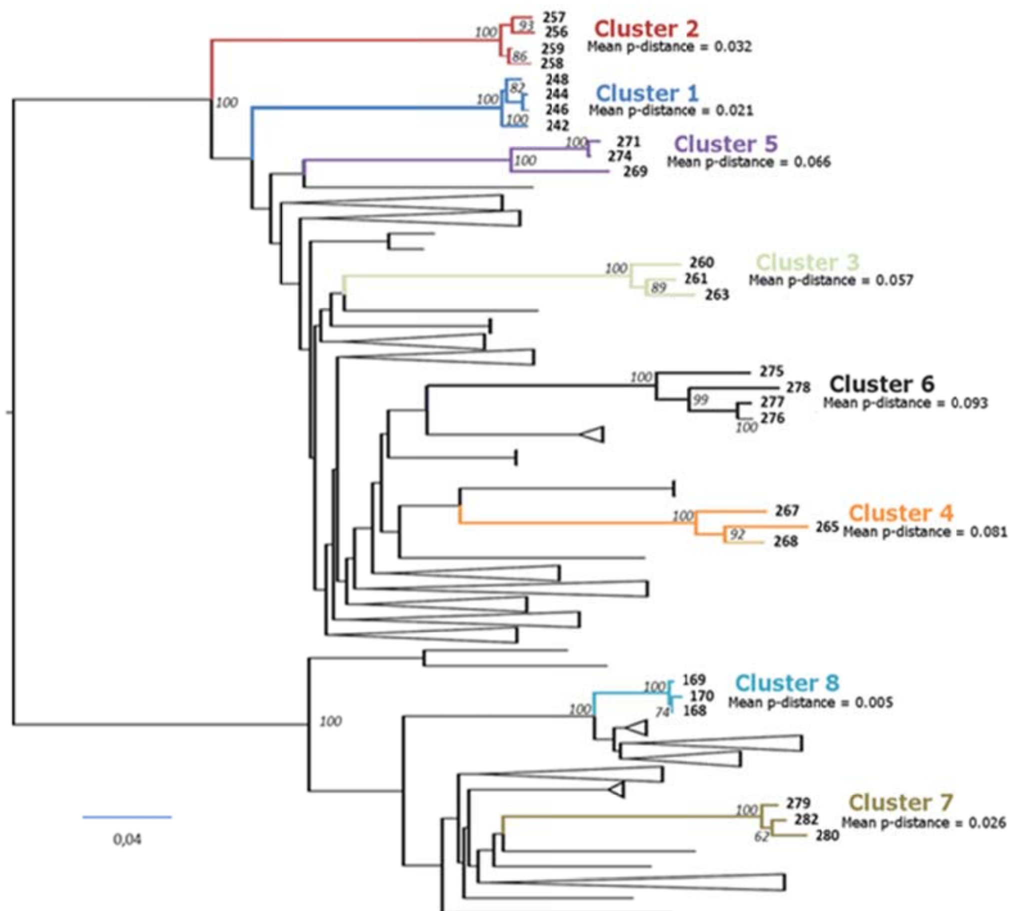

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Table 1: Length (aa number), number of PNGS and net charge of gp120 variable regions.

Cluster	Virus	V1			V2			V3			V4			V5		
		Length	PNGS	Charge	Length	PNGS	Charge	Length	PNGS	Charge	Length	PNGS	Charge	Length	PNGS	Charge
	HXB2	27	2	0	39	2	1	36	1	10	34	4	-3	10	1	-1
1	242	31	3	1	39	2	0	35	1	6	33	4	-6	10	2	0
	244	31	4	3	39	2	1	35	1	7	33	5	-3	10	2	0
	246	31	4	3	39	2	1	35	1	7	33	5	-3	10	2	0
	248	38	6	2	39	2	1	35	1	7	33	5	-3	10	2	0
2	256	23	2	0	47	3	1	35	1	5	30	4	0	12	2	-1
	257	24	2	0	45	3	0	35	1	5	30	3	-1	12	2	0
	258	27	3	-1	40	2	2	35	1	5	30	3	1	12	1	-1
	259	27	3	-1	45	3	3	35	1	5	30	3	1	12	2	-1
3	260	23	2	0	39	2	2	35	1	5	31	5	-2	10	1	-1
	261	26	3	0	41	2	1	35	1	6	39	7	-1	9	2	-1
	263	25	2	1	39	3	1	35	1	5	36	6	-4	9	1	0
4	265	31	4	-1	41	2	1	35	1	5	32	6	-2	14	2	0
	267	28	3	0	39	2	1	35	1	3	32	5	-1	11	2	0
	268	31	5	1	41	2	1	35	1	5	35	6	-2	14	2	-1
5	269	22	2	0	39	2	0	35	1	5	29	5	-1	10	1	-1
	271	30	3	-1	39	2	2	35	1	5	30	6	-2	10	1	0
	274	30	3	-1	39	2	2	35	1	5	30	6	-2	10	1	0
6	275	33	4	-1	38	1	3	35	1	3	34	5	0	9	1	-1
	276	35	4	-2	39	2	0	35	1	5	34	5	1	9	1	0
	277	35	4	-3	39	2	1	35	1	5	34	5	0	11	1	1
	278	35	5	0	39	2	0	35	1	7	36	4	-2	10	2	-1
7	279	15	1	1	44	2	2	35	1	4	31	3	2	10	1	-2
	282	12	1	-1	44	1	1	35	1	4	31	4	0	13	2	-1
	280	18	1	-2	44	2	1	35	1	4	29	5	0	10	1	-2
8	168	27	5	-1	41	2	3	35	1	3	35	6	-1	10	2	0
	169	27	5	-1	41	2	3	35	1	3	35	6	-1	10	2	0
	170	27	5	-1	41	2	3	35	1	3	35	6	-1	10	2	0

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Table 2: Sensitivity of transmitted viruses to neutralization by HuMoNAbs.

Cluster	Virus	CD4bs		V1V2		V3		MPER	gp120/gp41
		3BNC117 IC_{50} (g/mL)	NIH45-46[G54W] IC_{50} (g/mL)	PG9 IC_{50} (g/mL)	PGT145 IC_{50} (g/mL)	10-1074 IC_{50} (g/mL)	PGT121 IC_{50} (g/mL)	10E8 IC_{50} (g/mL)	8ANC195 IC_{50} (g/mL)
1	242	0.06	0.03	>10.0	0.23	< 0.005	< 0.005	0.39	1.72
	244	0.10	0.12	8.13	0.08	0.01	< 0.005	0.49	0.83
	246	0.09	0.11	7.66	0.12	0.01	< 0.005	0.9	1.28
	248	0.05	0.08	>10.0	0.14	0.03	0.02	0.74	1.76
2	256	0.22	0.08	0.33	0.04	0.09	0.06	0.25	0.35
	257	1.71	0.66	0.11	0.05	0.09	0.08	0.11	0.68
	258	0.85	0.29	0.63	0.06	0.11	0.11	0.07	0.89
	259	0.84	0.20	0.83	0.03	0.23	1.04	0.05	1.08
3	260	0.07	0.02	0.04	>10.0	0.01	< 0.005	0.54	>10.0
	261	0.09	0.02	2.10	>10.0	0.03	0.15	1.62	>10.0
	263	0.10	0.11	0.46	>10.0	0.01	0.42	0.61	>10.0
4	265	0.78	0.11	>10.0	0.24	0.06	0.03	0.29	>10.0
	267	0.08	0.02	6.58	0.40	0.03	0.01	0.38	>10.0
	268	0.66	0.09	>10.0	1.01	0.08	0.01	2.95	>10.0
5	269	0.51	0.31	0.11	0.01	0.02	0.01	0.1	1.04
	271	0.04	0.07	5.01	0.02	0.05	0.02	0.43	1.89
	274	0.06	0.08	1.64	0.01	0.02	0.01	0.31	1.26
6	275	0.36	0.01	>10.0	>10.0	>10.0	2.19	3.25	>10.0
	276	>10.0	8.35	2.25	2.54	>10.0	>10.0	2.02	>10.0
	277	>10.0	8.21	8.15	1.02	>10.0	9.28	0.71	>10.0
	278	>10.0	9.56	8.22	0.81	>10.0	>10.0	>10.0	>10.0
7	279	0.10	0.05	>10.0	>10.0	< 0.005	< 0.005	0.02	1.79
	280	0.31	0.17	>10.0	>10.0	0.04	< 0.005	0.12	2.35
	282	0.08	0.07	>10.0	>10.0	0.01	< 0.005	0.24	3.26
8	168	0.61	2.11	0.05	3.67	0.55	3.43	2.49	0.83
	169	0.36	2.61	0.09	6.21	0.28	2.21	1.1	0.35
	170	0.35	2.27	0.09	3.41	0.34	2.31	2.83	0.97

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Table 3: Sensitivity of transmitted viruses to entry inhibitors

Cluster	Virus	MVC		sCD4-183	sCD4	M48U1	T20
		IC50 (nM)	MPI (%)	IC$_{50}$ (g/mL)	IC$_{50}$ (g/mL)	IC$_{50}$ (g/mL)	IC$_{50}$ (g/mL)
1	242	0.52	95.5	> 50.0	> 50.0	< 0.005	0.67
	244	1.03	95.4	11.3	29.0	< 0.005	0.76
	246	1.00	99.0	17.0	43.1	< 0.005	0.31
	248	0.76	97.4	31.9	13.9	0.02	0.34
2	256	0.67	97.0	27.7	>50	0.33	0.58
	257	1.95	98.5	14.1	21.3	0.06	0.09
	258	1.09	97.8	9.80	11.3	0.34	0.16
	259	1.45	96.4	22.0	> 50.0	1.44	0.21
3	260	1.79	98.5	17.5	14.7	0.02	< 0.005
	261	1.05	99.0	17.8	> 50.0	0.01	< 0.005
	263	1.14	99.0	1.70	1.49	< 0.005	< 0.005
4	265	< 0.30	98.5	> 50.0	31.2	0.10	0.01
	267	< 0.30	99.9	12	11.4	0.01	0.02
	268	0.71	99.5	28.8	13.7	0.07	0.04
5	269	0.40	99.5	3.90	1.81	< 0.005	0.01
	271	1.03	99.7	12.2	1.16	< 0.005	0.01
	274	0.94	99.4	14.8	25.1	0.04	0.01
6	275	1.59	97.2	> 50.0	40.8	> 10.0	0.20
	276	1.10	98.5	12.0	12.5	0.94	0.14
	277	0.33	99.2	11.1	6.38	0.98	0.06
	278	1.33	98.5	3.70	1.39	0.33	0.08
7	279	0.99	96.4	38.0	26.3	0.40	0.01
	280	1.21	96.5	0.18	0.50	< 0.005	0.01
	282	1.28	98.3	40.0	14.4	0.29	0.02
8	168	1.25	97.5	22.0	40.5	0.06	0.48
	169	0.79	97.8	46.4	26.8	0.04	0.21
	170	1.25	99.3	31.9	36.8	0.10	0.49

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Table 4: Infectivity of transmitted viruses

Clusters	Virus	Infectivity ($\log_{10}$ RLU/25 ng P24	Mean $\log_{10}$ RLU/25 ng P24 (RSD)	Env content (ng Env/25 ng P24)	Infectivity ($\log_{10}$ RLU/1 ng Env)	Mean $\log_{10}$ RLU/1 ng Env (RSD)
1	242	6.52	6.65 (4.20)	0.6029	6.74	6.95 (2.56)
	244	6.41		0.2418	7.02	
	246	6.63		0.5680	6.88	
	248	7.05		0.7890	7.15	
2	256	6.46	6.25 (16.1)	<0.0002	na	na
	257	7.34		1.1827	7.26	
	258	4.90		0.0060	7.12	
	259	6.32		0.4422	6.68	
3	260	6.02	6.22 (4.50)	0.0876	7.07	7.14 (0.93)
	261	6.11		0.0910	7.15	
	263	6.54		0.2158	7.21	
4	265	5.79	6.23 (11.8)	0.2835	6.33	6.77 (9.40)
	267	5.83		0.2275	6.48	
	268	7.08		0.3811	7.50	
5	269	5.02	5.72 (10.7)	0.2465	5.62	5.99 (5.31)
	271	6.11		0.7789	6.21	
	274	6.05		0.8501	6.12	
6	275	7.44	6.24 (16.3)	<0.0002	na	na
	276	6.66		<0.0002	na	
	277	5.70		<0.0002	na	
	278	5.14		<0.0002	na	
7	279	5.49	5.59 (4.62)	0.0088	7.55	7.50 (1.66)
	280	5.89		0.0110	7.36	
	282	5.40		0.0194	7.60	
8	168	6.59	6.50 (2.41)	0.5768	6.83	6.77 (3.22)
	169	6.59		0.4430	6.95	
	170	6.32		0.6229	6.53	

RSD : relative standard deviation (%) ; na : not applicable

AC

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