315

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Frequent Envelope Recombination in Patients Superinfected or Co-infected with Distinct HIV-1 Subtype B Strains

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Background: Recombination is a common viral strategy that increases genetic diversity and optimizes adaptation in a more accelerated fashion than the gradual accumulation of mutations through replication errors. Fewer cases of intraclade recombination have been reported, probably due in part to the difficulty in detecting the recombinant sequences. Here we report detection of repeated recombination in the envelope gene of 4 of 5 patients infected with two strains of subtype B.

Methods: Longitudinal plasma samples were collected in five superinfection or coinfection cases from the UCSF Options cohort. All cases occurred in recent seroconverters. Envelope clones were isolated at each time point and sequenced to determine the composition of the virus quasispecies. The location and significance of the recombination crossover sites were estimated using Simplot software and the informative site analysis.

Results: Phylogenetic analyses based on cloned envelope sequences revealed that the two infecting strains in each patient were at least 10% divergent from each other and the differences were distributed over the entire length of gp160. Recombination events between the two viruses within the envelope gene were detected in multiple clones and found at different time points in 4 of 5 patients. The recombination frequency in the four patients was high, up to 100%. Most recombinations were observed in, or near, the conserved cytoplasmic tail region of gp41. In several clones, recombination occurred at more than one site in gp160.

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Table 1. Patient and virus characteristics

Patient	Estimated Infection Week	Co-Receptor Tropism	R5 (RLU)	X4 (RLU)	CD4 (cells/ul)	VL (copies/ml)	Observed Virus Population
1	131	R5	4,107,051	82	820	868	1st
1	166	R5	626,852	64	1271	1150	1st
1	177	R5	2,108,264	119	957	11000	2nd
1	272	R5	602,005	62	660	5111	2nd
2	29	R5	47,907	59	561	10525	1st
2	33	R5	35,727	65	570	77827	2nd
2	39	R5	22,431	67	400	304353	2nd
2	44	R5	31,775	66	350	433000	2nd
2	81	DM	708,248	54,901	247	447037	2nd(R5+DM)
2	100	DM	256,791	6,649	37	>500000	2nd(R5+DM)
3	43	DM	240,612	996,311	585	522	1st
3	53	DM	1,530,233	182,765	439	158796	1st+2nd
3	59	DM	112,573	282,507	490	4226	1st
3	114	DM	156,385	220,745	388	127733	1st+2nd
4	10	R5	1,897,836	62	675	4158	1st
4	14	R5	211,181	84	690	399	1st
4	99	R5	15,305	63	672	4698	2nd
4	237	R5	9,686	70	384	1321	2nd
5	10	R5	75,965	57		81,392	1st
5	13	R5	373,527	64		126,914	2nd
5	22	R5	99,994	69		47,391	1st+2nd
5	42	R5	132,891	60		90,223	2nd

RLU represents Relative Light Unit; R5 is CCR5 tropic; DM is dual/mixed CCR5/CXCR4 tropic.
Co-receptor tropisms were determined using the PhenoSense HIV Entry Assay. The virus is designated CCR5 if it can infect U87/CD4CCR5R5. DM tropism indicates the virus could infect both U87/CD4/CXCR4 and U87/CD4/CCR5 cells.
Observed virus population indicates whether original infecting virus, called 1st, or emergent/superinfection virus, called 2nd, is found in the clones picked form the virus population at each timepoint. This may not reflect the full range of virus clones present in the population.

Table 2. Genetic distance of initial virus and superinfecting virus

Patient	Genetic diversity(%) V1	V2	Between V1 and V2	Between V1 and control	Between V2 and control
1	0.79 (0.0 - 1.4)	1.19 (0.0 - 1.8)	11.47 (11.1 - 12.0)	11.29 (8.4 - 13.6)	11.19 (9.0 - 13.5)
2	0.38 (0.3 - 0.5)	2.38 (0.0 - 4.2)	9.87 (8.9 - 11.3)	9.06 (7.2 - 11.2)	11.54 (9.5 - 13.9)
3	0.56 (0.3 - 0.9)	0.33 (0.0 - 0.6)	11.60 (10.5 - 12.2)	10.93 (8.3 - 13.5)	11.48 (8.4 - 13.6)
4	0.30 (0.0 - 0.5)	1.37 (0.0 - 2.3)	10.99 (10.4 - 11.9)	10.43 (7.7 - 13.2)	10.71 (7.9 - 13.4)
5	0.54 (0.0 - 1.7)	0.15 (0.0 - 0.4)	12.12 (11.7 - 12.4)	11.35 (9.6 - 13.7)	10.71 (8.7 - 13.4)

Pairwise distances were calculated on aligned sequences with the variable regions (V1-V5) removed for virus 1 (initial virus) and 2 (superinfecting virus) populations, and the controls which consisted of 48 randomly selected gp160 sequences from the Los Alamos National Laboratory and HXB2 using Mega 3.1.

Table 3 . Recombinant rate and crossover site

Patient	Week after superinfection	Clone number	Observed recombinant number	Recombinants in different crossover site: C1	V3 loop	gp120/41 cleavage site	Transmembrance region	Cytoplasmic Tail
1	0	10	5 (50%)	0	0	0	1	4
1	95	14	0	0	0	0	0	0
2	0	7	1 (14.3%)	0	0	1	0	
2	6	12	10 (83.3%)	6	0	1	0	9
2	11	12	12 (100%)	4	0	0	0	10
2	48	10	6 (60%)	5	0	0	0	2
2	67	18	0					
3	0	14	2 (14.3%)	1	0	0	0	2
3	6	11	6 (54.5%)	6	0	0	0	6
3	61	18	14 (77.8%)	14	0	0	0	14
4	0	9	0	0	0	0	0	0
4	138	10	0	0	0	0	0	0
5	0	8	0	0	0	0	0	0
5	9	14	8 (57.1%)	3	1	0	0	7
5	29	15	15 (100%)	8	1	1	0	14
Total		182	79 (43.4%)	47	2	3	1	68

Patient 5 is a coinfection patient. We could not detect the superinfecting virus at the first time point. Week 0 in this table means is the time that the superinfecting virus was first detected.

Figure 1. Neighbor joining tree

Neighbor joining tree was constructed from the longitudinal gp160 sequences from longitudinal samples of each patient and the reference strain, HXB2. Sequences from the initial infecting virus are labeled green color , superinfecting sequences are labeled by orange color and recombinants are labeled by black color. Bootstrap values are shown at nodes. The scale bar represents genetic distance(1=100% divergence).

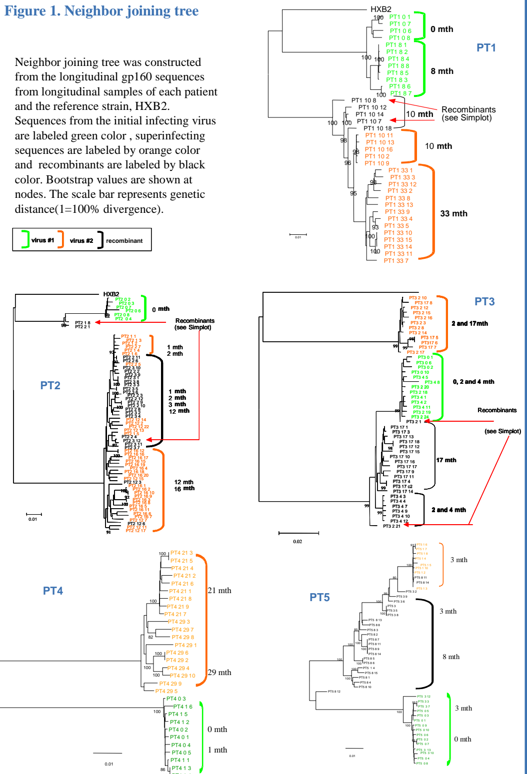
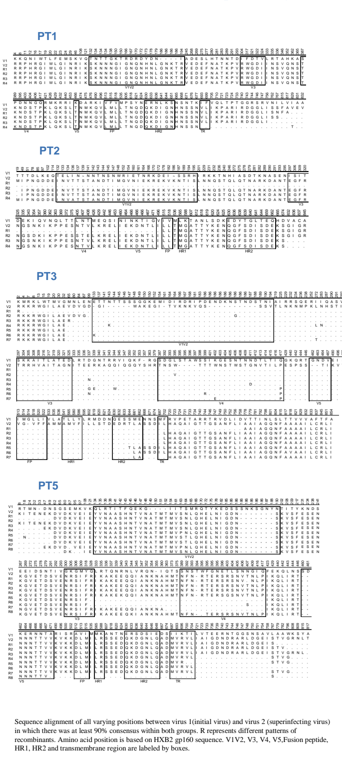
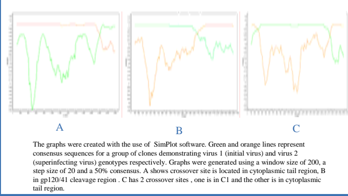


Figure 2. Patient amino acid sequence alignment



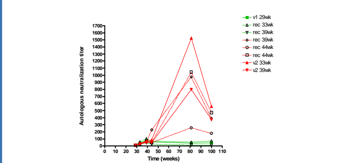
Sequence alignment of all varying positions between virus 1(initial virus) and virus 2 (superinfecting virus) in which there was at least 90% consensus within both groups. R represents different patterns of recombinants. Amino acid position is based on HXB2 gp160 sequence. V1V2, V3, V4, V5,Fusion peptide, HR1, HR2 and transmembrane region are labeled by boxes.

Figure 3. SimPlots for recombinants



The graphs were created with the use of SimPlot software. Green and orange lines represent consensus sequences for a group of clones demonstrating virus 1 (initial virus) and virus 2 (superinfecting virus) genotypes respectively. Graphs were generated using a window size of 200, a step size of 20 and a 50% consensus. A shows crossover site is located in cytoplasmic tail region, B in gp120/41 cleavage region . C has 2 crossover sites , one is in C1 and the other is in cytoplasmic tail region.

Figure 4. Autologous neutralization titers of patient 2 plasma against initial, superinfecting and recombination virus



Autologous neutralization titers of patient 2 plasmas against initial, superinfecting and recombinant viruses. Neutralizing antibody titers are expressed as the reciprocal of the dilution of plasma required to reduce the viral replication rate to 50% relative to a negative control with not plasma.

Conclusions: Envelope recombination events were identified by envelope sequence analysis in 4 out of 5 subtype B infected individuals with super-infection or coinfection. The cytoplasmic tail region was identified as a "hot spot" for recombination in these viruses. Recombination may be observed at these sites because of genetic conservation, or because recombination allows for immunological escape while preserving viral genetic determinants that are important in primary HIV infection or superinfection.

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