

Long Term Multicompartment Evolutionary Study of HIV-*env* in a Chronically HIV Infected Patient Before and During HAART Followed by 5 Structured Treatment Interruptions (STI)

Abstract

Background: Extensive study on HIV *env* sequence evolution over 5 years in patient undergoing multiple STI cycles and induction of host immune responses.

Methods: Patient history: Infected with HIV-1B without therapy for ≥ 6 years. Successful ART for 34 months. Enrolment into Swiss Spanish Intermittent Therapy Trial by wk0. 4 STI cycles (2 wk off, 8 wk on ART). ART interrupted at wk40 and restarted at wk50. Specimen collection: Plasma and PBMC before any ART, on ART and during STI (wk0,2,10,12,20,22,30,32,40,42,49). Tonsil biopsies before ART. Virus culture supernatants (wk2,41). Analyses: HIV-RNA subjected to RT-PCR, cloning (15-20 clones/sample), and sequencing of *env* C2V3C3 domain. 400 sequences analyzed (PHYLIP, Mega).

Results: A diverse spectrum of HIV *env* quaspecies with several distinct phylogenetic groups was present prior to ART. Cluster A, predominant in pre-ART tonsils and PBMC (30/47 clones), persisted during continued ART (14/16, PBMC wk0) and comprised the rebounding virus in plasma during the 1st STI (16/16, wk2). During later STI cycles, this population was cleared. Plasma virus rebounding during STI (wk12-49) belonged to a uniform cluster B which was also present in pre-ART plasma, PBMC and tonsils. Autologous virus isolated at wk2 from PBMC belonged to cluster B. The neutralizing activity of patient plasma against B and the nonsynonymous vs synonymous mutations ratio in clonal plasma *env* sequences continuously increased in parallel after the 2nd STI (wk12-49). This indicates selective pressure on the *env* gene most likely due to induction of neutralizing antibodies. HIV-specific CTL against 5 epitopes were detectable before, declined to low levels on ART and were partially reinduced during STI.

Conclusion: HIV populations emerging during successive STI may reflect both stochastic reactivation of archived subpopulations and ongoing selection due to host antibody and CTL responses. Pretreatment viral diversity and continued co-evolution of virus and host immune responses appear to be important factors in restricting replicating viral subpopulations during STI.

Objective

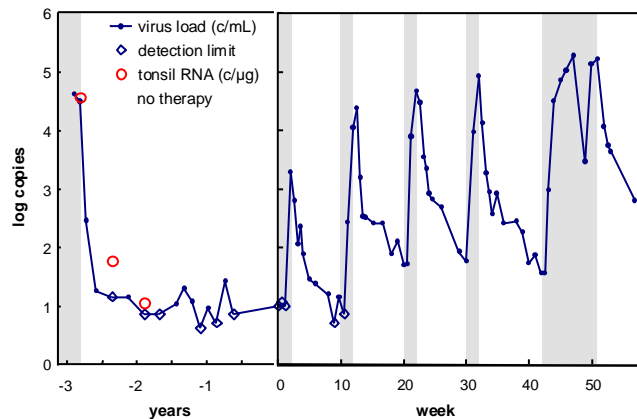
- To determine phylogeneticity of HIV rebounding during STI with regards to quaspecies preexisting before any therapy.
- Longitudinal study of evolution of HIV *env* sequences and induction of host immune responses in a patient who underwent multiple STI cycles.

Methods

Patient:

- was infected with HIV-1B without therapy for ≥ 6 years.
- had successful ART for 34 months (AZT, 3TC, ritonavir).
- was included in Swiss Spanish Intermittent Therapy Trial (SSITT) consisting of 4 STI cycles (2 wk off, 8 wk on ART).
- finally ART was interrupted at wk40 and restarted at wk50.

Sampling:

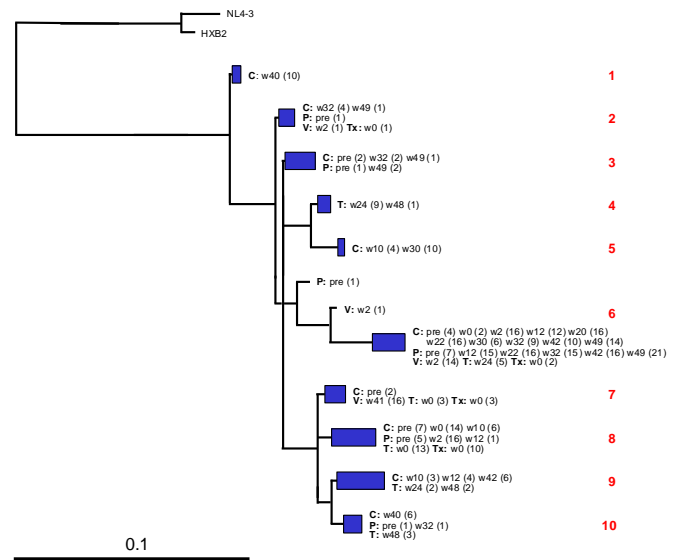


Plasma:	✓	-✓	-✓	-✓	-✓	-✓	✓
PBMC:	✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓
Tonsils:	✓	✓	✓				
Culture supernatants:		✓					✓

Analyses:

- Extraction of HIV-RNA (Qiagen Qiamp or RNeasy).
- RT-PCR amplification (Finnzyme RobusT RT-PCR) followed by nested PCR (Qiagen HotStar Taq).
- Cloning by Invitrogen pCR4-TOPO (15-20 clones/sample).
- Bidirectional ABI sequencing of the *env* C2V3C3 domain.
- Phylogenetic analyses of a total of 400 sequences: inferred trees by maximum likelihood method (PHYLIP) genetic distances by Tamura Nei 6-parameter model (Mega).

Results



Horizontal bars represent diversity of descending quaspecies. P = plasma, C = cells (PBMC), V = virus culture supernatant T = tonsil, Tx = extracellular virus from tonsil
Note: Only highly significant branches ($p < 0.01$) are shown.

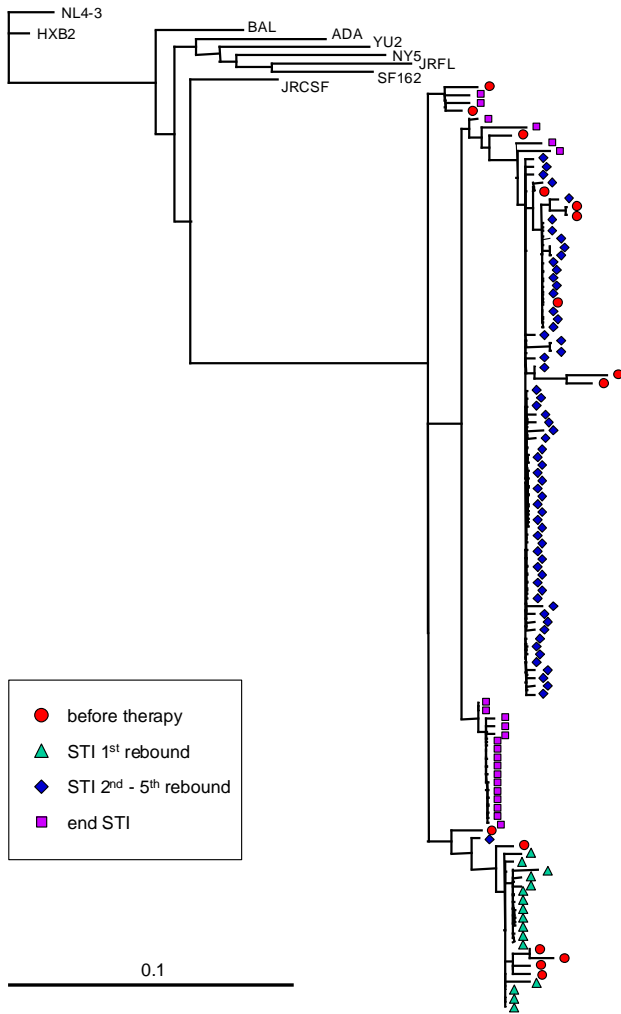
	Cluster										Diversity (%)	Divergence* (%)	
	1	2	3	4	5	6	7	8	9	10			
Plasma													
Pre		1	1			8		5		1		3.92	
wk 2									16			0.58	1.34
wk 12						15			1			0.92	0.90
wk 22						16						0.36	0.99
wk 32						15				1		0.23	1.02
wk 42						16						0.50	1.03
wk 49			2			21						1.63	1.28
PBMC													
pre			2			4	2	7				3.58	
wk 0						2		14				1.67	0.82
wk 2						16						0.55	2.13
wk 10 ^s					4			6	3			3.26	0.67
wk 12						12			4			2.86	1.04
wk 20						16						0.25	1.98
wk 22						16						0.26	2.01
wk 30						10	6					3.37	1.11
wk 32 ^s			4	2		9						2.62	0.66
wk 40										6		2.38	1.61
wk 42										6		3.51	0.94
wk 49 ^s		1	1									1.99	1.18
Culture													
wk 2						15						1.10	
wk 41								16				0.36	3.86
Tonsils													
wk 0 [†]							3	13				2.60	
wk 0 ^{extra}		1					2	3	10			3.01	0.15
wk 24 [†]				9			5			2		3.18	1.61
wk 48 [†]				1						2	3	2.34	1.38

* net divergence from initial (%)

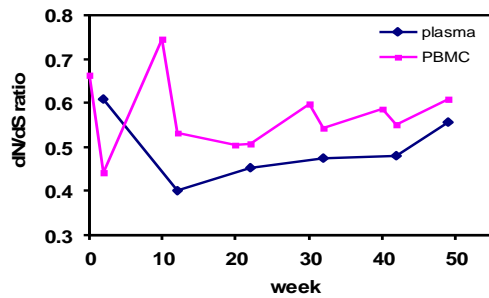
^s eight PBMC sequences were excluded due to possible contamination

[†] weeks after beginning of first antiretroviral therapy

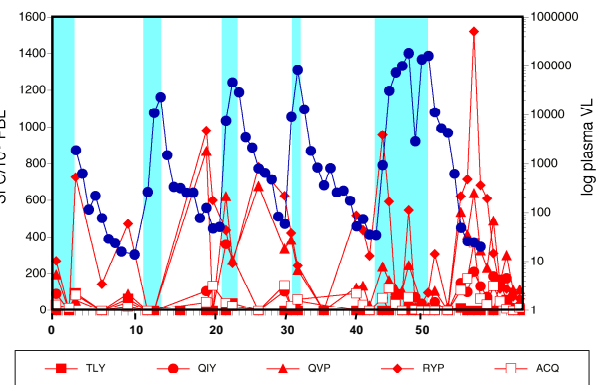
Plasma Sequences:



Nonsynonymous / synonymous mutations:



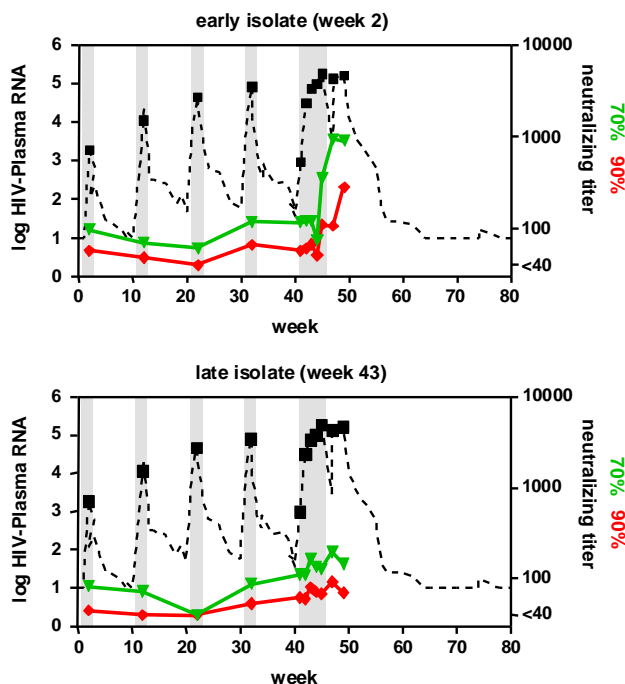
HIV-specific CTL:



Summary

- A diverse spectrum of HIV *env* quasispecies with eight distinct phylogenetic groups was present prior to STI.
- The predominant cluster (8) in pretreatment tonsils and PBMC (30/47 clones) persisted during continued therapy (14/16 clones in PBMC at week 0) and comprised the rebounding virus in plasma during the 1st STI (16/16 clones, week 2).
- This population was completely cleared from all compartments. during later STI cycles.
- Plasma virus rebounding by week 12-49 belonged to a uniform cluster (6) which was also present in pre-ART plasma, PBMC and tonsils.
- Autologous virus isolated at week 2 from PBMC belonged to cluster 6.
- The neutralizing activity of patient plasma against 6 and in parallel the nonsynonymous vs. synonymous mutations ratio in clonal plasma *env* sequences continuously increased after the 2nd STI (week 12-49).
- This indicates selective pressure on the *env* gene most likely due to induction of neutralizing antibodies.
- HIV-specific CTL against 5 epitopes were detectable before, declined to low levels on ART and were partially reinduced during STI.

Neutralization assay:



Conclusions

- HIV populations emerging during successive STI may reflect both stochastic reactivation of archived subpopulations and ongoing selection due to host antibody and CTL responses.
- Pretreatment viral diversity and continued co-evolution of virus and host immune responses appear to be important factors in restricting replicating viral subpopulations during STI.