

Abstract

Background: Genetic diversity of viral isolates in HIV-infected individuals varies substantially. However, it remains unclear whether HIV-related disease progresses more rapidly in patients harboring virus swarms with low or high diversity and, in the same context, whether high or low diversity is required to induce potent humoral and cellular immune responses.

Methods: To explore whether viral diversity predicts virologic control, we studied HIV-infected patients, who received antiretroviral therapy (ART) for years before undergoing structured treatment interruptions (STI). HIV *env* gene diversity before initiation of ART and the ability of the patients to contain viremia after STI and final cessation of treatment was evaluated. Moreover, *in vitro* replication capacity, proviral DNA load in peripheral blood mononuclear cells (PBMC), HIV-specific cytotoxic T-lymphocyte or cytotoxic T-cell (CTL) and T-helper responses, neutralizing antibody response against autologous virus, host genetic factors associated with the course of HIV disease (CCR2 [V64I], CCR5 [G-2455A], CCR5 [delta 32], CX3CR1 [T280M], interleukin (IL)-10 [C-592A], RANTES [G-403A], RANTES [C-28G], MIP-1 α [T113C], SDF-1 [3'A], and HLA types [HLA-A*02, A*6802, A*11, B*27, B*51, B*57, B*58, Cw*08 and DRB1*01, HLA- A23, B*08, B*3501, B*45, B*53, Cw*04]), and HIV genotypic drug resistance were determined.

Results: Seven out of 21 patients contained plasma viremia at low levels after the final treatment cessation. Clonal sequences encompassing the Env C2V3C3 domain derived from plasma prior to treatment exhibited significantly lower diversity in these patients compared to those derived from patients with poor control of viremia. Viral diversity pre-ART correlated with viral replication capacity of rebounding virus isolates during STI. Neutralizing antibody activity against autologous virus was significantly higher in patients who controlled viremia and was associated with lower pre-treatment diversity. No such association was found with binding antibodies directed to gp120. In contrast, HIV- specific CD8⁺ and CD4⁺ T-cell responses did not correlate with diversity and showed no differences between controllers and non-controllers and no associations between viral diversity, control of viremia, and host genetic factors.

Conclusions: In summary, lower pretreatment viral diversity was associated with spontaneous control of viremia, reduced viral fitness, and higher neutralizing antibody titers, suggesting a link between viral diversity, viral fitness, and neutralizing antibody activity.

Objective

- To study plasma HIV-*env* diversity in HIV-infected patients prior to long term combination antiretroviral therapy (ART).
- To test whether pretreatment diversity predicts virological control after STI and correlates with viral replication capacity and/or with cellular or humoral immune responses.
- To investigate whether virological response is influenced by host genetic factors.

Patient characteristics

Patient	Age (years)	Gender ^a	HIV-1 infection (months)	HIV subtype	Coreceptor usage of viral isolates (5 th STI cycle)	ART received before STI ^b	HIV RNA before ART (copies/ml)	viral diversity before ART (%)	duration of ART before STI (months)	HIV RNA post STI plateau (copies/ml)
control of viremia										
106	35	m	>24	B	R5	AZT, 3TC	12'000	0.71	37	5128
107	42	f	>24	B	R5	AZT, 3TC, NFV	5'216	2.12	32	2551
112	42	m	6-12	B	R5	AZT, 3TC, IDV	27'100	1.61	32	6866
117	30	f	12-24	B	R5	AZT, 3TC, RTV	29'522	0.88	40	13007
118	29	m	12-24	B	R5	AZT, 3TC, IDV	16'927	0.94	34	3099
123	36	f	>24	B	R5	AZT, 3TC, RTV	14'410	2.87	37	118
124	41	m	12-24	B	R5	ddl, d4T, NFV	22'464	2.93	20	6218
no control of viremia										
101	39	m	>24	B	R5	ddl, d4T, NFV	10'752	5.51	20	32607
103	41	m	>24	E/CRF1	R5	ddl, d4T, NFV	9'758	2.81	16	102953
109	34	m	>24	B	R5	AZT, 3TC, RTV	42'277	4.02	34	148191
111	32	m	>24	B	R5	ddl, d4T, NFV	129'408	4.66	17	22030
113	56	m	12-24	B	R5	AZT, 3TC, RTV	107'303	2.27	35	34110
114	29	m	>24	B	R5	AZT, 3TC, RTV	9'275	2.74	35	28478
115	22	m	12-24	B	R5	D4T, 3TC, NFV	15'118	1.60	26	9368
119	34	m	12-24	B	R5	D4T, 3TC, SQV, RTV	98'561	1.60	18	99274
120	51	m	12-24	B	R5	AZT, 3TC, IDV	100'618	2.97	30	38252
121	35	m	3-6	B	R5	D4T, 3TC, NFV	164'772	1.23	21	67321
122	38	m	>24	B	R5	AZT, 3TC, IDV	12451	3.34	33	24982
126	46	m	>24	B	R5	AZT, 3TC, RTV	63'698	3.87	39	19782
127	48	f	>24	B	R5	D4T, 3TC, NFV	25'417	2.95	26	106923
128	39	f	>24	B	R5	AZT, ddl, NFV	9'404	4.90	29	20236
Median: control of viremia							16927	1.61	34	5128
Median: no control of viremia							33847	2.96	28	33359
p-value (Mann-Whitney)							0.31	0.023	0.093	0.0004

^a m = male, f = female

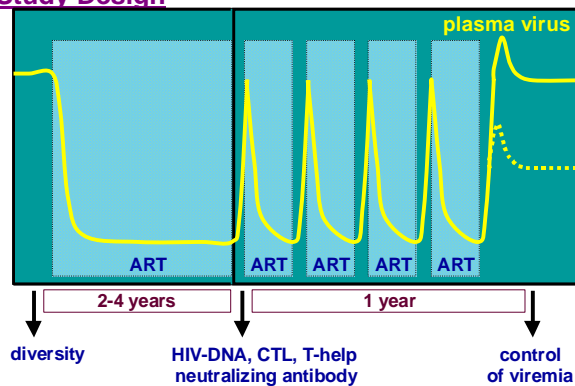
with *in vitro* Replication Capacity and Predicts Plasma Viremia after Treatment Interruptions.

Methods

- plasma collected from 21 HIV infected patients before successful ART during 2 - 4 years and subsequent STI
- HIV-RNA extracted, RT-PCR, cloning (16 clones/sample) and sequencing of the *env* C2V3C3 domain
- phylogenetic analyses with PHYLIP 3.6 and Mega 2.0
- replication capacity from autologous virus week 42-50
- neutralization activity in patient plasma directed against autologous virus measured in a standard PBMC neutralization assay
- anti-gp120 Ab titers in patient plasma determined by measuring reactivity against gp120 from strain JR-FL in an ELISA assay
- HIV-specific CTL measured by gamma interferon ELISpot
- control of viremia defined as plasma viral load <5000 RNA copies/mL for at least 2 months after treatment stop
- Host-genetic factors (HLA typing and CCR-polymorphisms)
- HIV-drug resistance (genotyping) during and after last rebound

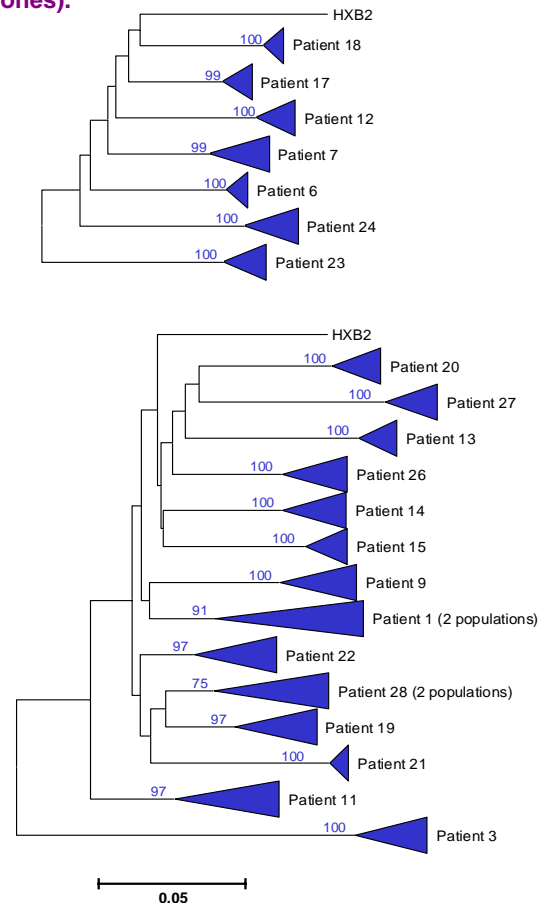
Def. Controllers: Patients controlling viremia spontaneously after last STI between week 40 and 76 (8 weeks or more < 5000 RNA copies/ml between week 40 and 76 of SSITT).

Study Design

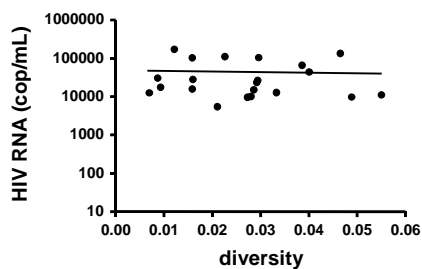


Results

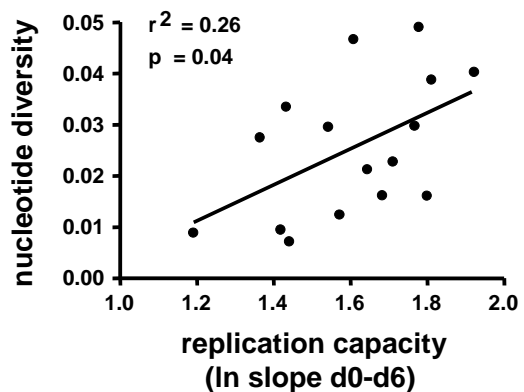
Inferred neighbor-joining phylogenetic trees of clonal C2-C3 sequences from 7 controllers and 14 non-controllers before treatment (1 triangle = 16 clones).



There was no correlation between viral load and diversity, suggesting that sampling had no influence on diversity measurement.



A significant positive correlation between replication capacity after the last STI and pretreatment *env* diversity was found.



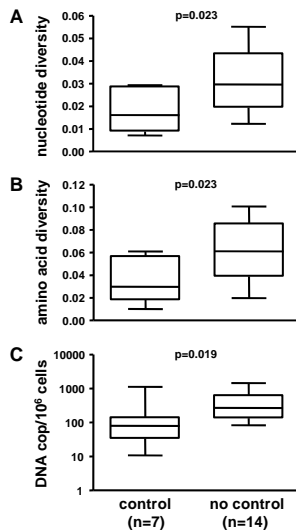
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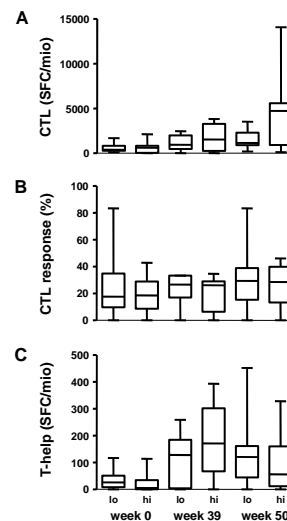


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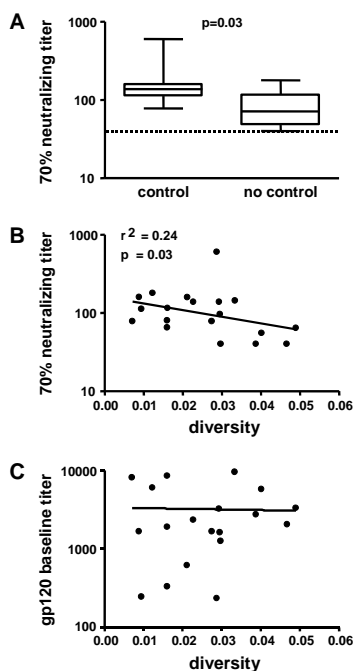
Nucleotide and amino acid diversity pre-ART and baseline proviral DNA was lower in controllers than in non-controllers.



Neither breadth and magnitude of HIV-specific CD8 T-cell response nor magnitude of HIV-specific T-help response was correlated with pre-treatment diversity.



Plasma neutralizing activity against autologous virus was higher in controllers than in non-controllers and correlated with pre-treatment diversity.



HLA and host genetic factors associated with faster disease progression (see abstract) were not found at a higher frequency in controllers (data not shown).

Summary

- Lower pretreatment viral diversity in patients with stronger spontaneous control of viremia after treatment stop.
- Lower in vitro replication capacity of viral isolates from patients with low pre-ART diversity.
- Lower HIV DNA levels at baseline of STI in PBMC of controllers.
- Higher plasma neutralization activity against autologous virus in patients controlling viremia.
- Higher neutralizing activity associated with lower pretreatment diversity.
- Anti gp120 titers, HIV specific CTL-, and T-help-responses not associated with diversity.

Lower pretreatment *env* diversity was associated with spontaneous control of viremia, reduced replication capacity and higher neutralizing antibody titers.

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