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Improved Antibody Cross-Neutralizing Activity in HIV-1 Dual-Infected LTNP Patients

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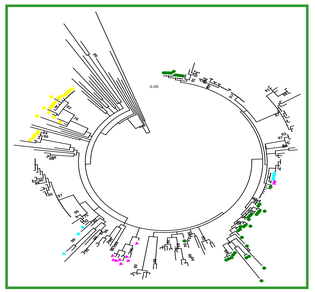
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Background

HIV-1 infected individuals, classified as long-term non-progressors (LTNP), remain healthy for many years in absence of antiretroviral therapy, with stable CD4+ T lymphocyte counts and low levels of viral replication. Neutralization studies in sera from LTNPs with undetectable viremia, showed little neutralizing activity (Nab) because of a reduced antigenic stimulation of B cells. A broad NAb response has been associated with prolonged high-levels of antigenic stimulation, in terms of high viral load and viral diversity. In fact, patients infected with two different HIV-1 variants showed enhanced heterologous NAb response. However, little is known about the neutralizing response in LTNP patients with dual infection (DI).

Methods

Detection of DI



LTNP´s sequences in the C2-V5 region in the env gene were obtained by single genome amplification and subjected to phylogenetic analysis (Casado et al; 2007, Pernas et al; 2013). DI was defined when *env* sequences of the same patient were segregated in two or more different clusters of the ML phylogenetic tree.

Figure1 . ML Phylogenetic tree in the C2-V5 env region. Sequences of LTNP, chronic progressor Spanish patients and two reference subtype B were included in the analysis. A subtype D sequence D.CD.83.ELI (★) was included as out-group. Bootstrap values above 80% are marked. DI patients are marked (MDM ●, LTNP-5 ■, LTNP-12 ▼and LTNP-15 ■). Bar corresponds to genetic distance.

Patients

Heterologous Nab response was compared in 4 DI versus 6 single infected LTNP patients.

Table 1. Patient characteristics

Patient	Patient classification	First HIV-1 +	Viral load (copies/ml)	CD4+ T cells (cells/ul)
Dual infected patients				
LTNP-15	LTNP-VC	1989	253	703
LTNP-5	LTNP-EC	1986	50	842
MDM	LTNP-EC	1988	50	1009
LTNP-12	LTNP-VC	1986	2970	509
Median		**1987**	**378**	**635**
Single infected patients				
LTNP-20	LTNP-EC	1985	51	894
LTNP-1	LTNP-VC	1996	125	746
LTNP-2	LTNP-EC	1999	53	1387
LTNP-3	LTNP-EC	1988	50	850
LTNP-17	LTNP-EC	1987	50	1354
LTNP-21	LTNP-EC	1992	50	487
Median		**1990**	**50,5**	**872**

I. All patients met the LTNP criteria, more than 10 years since the first HIV-1 detection without clinical symptoms in absence of therapy.
II. Patients were classified as LTNP-VC (mean viral loads< 10,000 copies/ml) or LTNP-EC (patients viral loads <50 copies/ml).
III. DI and single-infected LTNP patients were matched according to viral loads, T CD4+ cell counts and year of first HIV-1 + after HIV-1 diagnosis.

Neutralization assays

Two samples taken at different times were tested against a mini-panel of six recombinant viruses from 5 different subtypes and tropisms that adequately represented the global HIV-1 diversity (Medina et al; 2011). Nab breadth, expressed as number of subtypes crossed, was also estimated.

Analysis of quasispecies viral evolution

A mean of 16 sequences (range 6-27) in the C2-V5 env region were obtained in samples taken during the clinical follow-up (range 4-5). Shannon entropy was calculated, as an estimation of DNA viral diversity, with the Entropy one tool available in www.hiv.lanl.gov. A correlation between Shannon entropy and subtypes crossed was estimated by using Graph Pad V5.1.

Results

Figure 2. Heterologous neutralization of Dual/ Single infected patients.

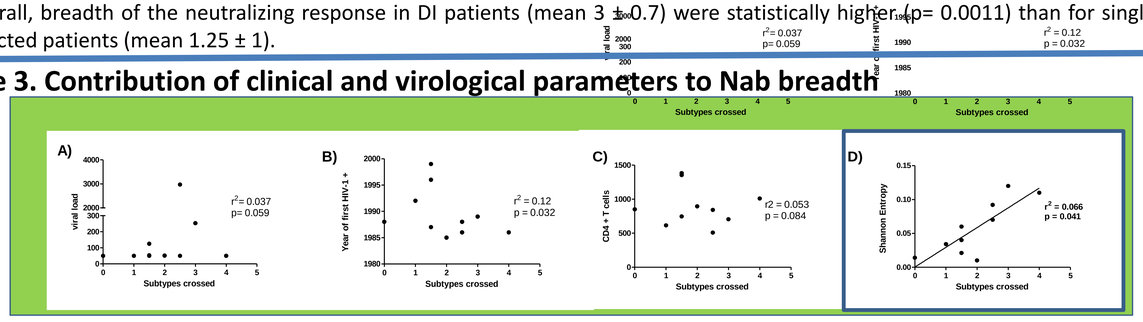
		VIRUS							
Dual Infected	**Sample**	**V1 191 (A) (T2)**	**NL4-3 (B) (T1)**	**AC10 (B) (T2)**	**92BR025 (C) (T1B)**	**92UG024 (D) (T2)**	**CM244 (E) (T2)**	**VSV**	**Subtypes Crossed**
	LTNP 15-1	69.2 ± 12	27.2 ± 2	33 ± 3.5	40.6 ± 5.2	70.6 ± 2.5	38 ± 4.6	102.2 ± 19.8	3
	LTNP 15-3	58.2 ± 8.8	29.8 ± 14.6	26.3 ± 4.3	28.9 ± 2.5	61.1 ± 5.3	39.7 ± 6.5	111.1 ± 4.3	3
	LTNP 5-1	110 ± 9.6	22.9 ± 18.2	75.9 ± 13.4	43.5 ± 4.4	72.6 ± 0.5	62.5 ± 8.5	110 ± 1.6	2
	LTNP 5-9	100.4 ± 11.3	31.4 ± 11.2	79.9 ± 16.7	44.4 ± 16.2	74.9 ± 4.2	42.7 ± 1.6	100.4 ± 11.3	3
	LTNP 12-1	66.6 ± 0.8	35.2 ± 10.6	87.5 ± 4.8	43.6 ± 19.4	73.7 ± 3.7	46.8 ± 6.9	116.3 ± 10.8	3
	LTNP 12-13	101.8 ± 9.7	31 ± 8.9	78.7 ± 9.1	20.1 ± 4.1	60.8 ± 2.3	53.9 ± 8.1	101.8 ± 4.3	2
	MDM -95	21.8 ± 4.1	5.8 ± 1.6	106.2 ± 26.2	27.4 ± 1.9	38.9 ± 4.8	75.8 ± 11.5	84.5 ± 7.7	4
	MDM -98	12.9 ± 1.4	4 ± 5.0	99.9 ± 15.2	24.4 ± 1.8	31.3 ± 1.3	73.1 ± 7.8	92.8 ± 14.3	4
									Median 3±0.7
Single Infected	LTNP 20-1	65 ± 5.9	27.5 ± 6.1	100.9 ± 4.5	41.1 ± 6.8	74.9 ± 3.8	50.2 ± 13.1	103.1 ± 5	2
	LTNP 20-5	114.3 ± 12.1	22.1 ± 13.7	93.8 ± 11.1	45.6 ± 3.8	59.8 ± 12.3	53.7 ± 18.9	114.31 ± 12.1	2
	LTNP 1-1	80.2 ± 5.2	57.2 ± 12.5	95.8 ± 12	83.3 ± 5.9	102.3 ± 6.6	68.2 ± 7.5	110.4 ± 4.1	0
	LTNP 1-7	65.4 ± 8.5	24.2± 11.5	75.3 ± 16	51.9 ± 3.8	83.9 ± 12	47.1 ± 2.7	115.1 ± 7.3	2
	LTNP 17-1	86.5 ± 10.9	40.5 ± 10	68.4 ± 10.2	45.3 ± 2.6	38.5 ± 0.5	77 ± 8.2	86.5 ± 10.9	3
	LTNP 17-4	125.7 ± 10.3	68.9 ± 5.5	80.4 ± 4.4	93.7 ± 9.4	102.5 ± 11.2	82.5 ± 8.2	125.7 ± 10.3	0
	LTNP 3-1	113.6 ± 7	57.3 ± 12.3	79.2 ± 6.9	59.6 ± 4.3	104.5 ± 8.4	66.1 ± 18.7	105.9 ± 6.5	0
	LTNP 3-9	106.6 ± 4.1	62.6 ± 16	65.5 ± 3.7	73.2 ± 3.9	103.5 ± 5.8	92.2 ± 9.2	106.6 ± 4.1	0
	LTNP 2-1	114.1 ± 3	41.6 ± 11.9	89.9 ±	61.8 ± 3.8	91.9 ± 3.3	72.3 ± 11.9	114.1 ± 3	1
	LTNP 2-4	64.6 ± 11.5	31.2 ± 7.8	87.7 ± 18.3	56.6 ± 3.8	76.6 ± 6.4	46.7 ± 7.9	112.1 ± 6.6	1
	LTNP 21-2	101.4 ± 10.6	67 ± 10.5	94.6 ± 8.5	71 ± 7.8	94.9 ± 11.8	66.4 ± 6.4	101.4 ± 10.6	0
	LTNP 21-6	102.7 ± 2.9	40.8 ± 12	87.3 ± 12	60.7 ± 8.5	81.9 ± 2.7	41.6 ± 4.7	102.7 ± 2.9	2
									Median 1.25±1

< 10%
10 - 30%
30 - 50%
> 50%

Values represent % of infection after neutralization. In brackets is shown subtype virus and the tier response.

- All the DI patients showed a broad (defined as response to 4 or 5 isolates) or intermediate response (response to 2 or 3 isolates)
- Overall, breadth of the neutralizing response in DI patients (mean 3 ± 0.7) were statistically higher (p= 0.0011) than for single-infected patients (mean 1.25 ± 1).

Figure 3. Contribution of clinical and virological parameters to Nab breadth



- No correlation was observed between viral load (A), year of first HIV 1+ (B) nor CD4+ T cells (C) and the number of subtypes crossed.
- A high positive correlation (p= 0.084, r^2 = 0.66) (Figure 3 D) between viral diversity and the number of subtypes crossed was observed, suggesting that viral diversity has contributed to the increase of Nab breadth.

Conclusions

- **We found a positive correlation between the neutralization breadth and diversity within viral quasispecies generated by DI in LTNP patients.**
- **Our results support the hypothesis that, in LTNP patients with low levels of viremia, viral diversity within the quasispecies because of DI may be a driver of the neutralization breadth.**

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