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Viral Evolution Analysis for the Distinction of True HIV-1 Elite-Controller Patients

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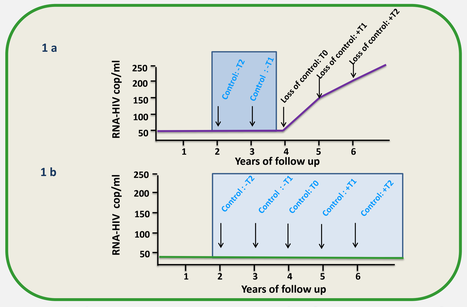
Background

Elite controllers (EC) are a sub-set of HIV-infected patients able to spontaneously maintain viral loads below the limit of detection without therapy. Some EC undergoes events of viral replication, while others remain with undetectable viral load and they could be considered as the true elite controllers. The distinction between both groups of EC is of major importance. In this work, we propose to study viral evolution as a marker to distinguish true EC.

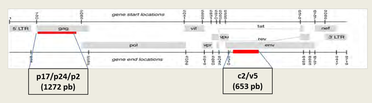
Methods

Patients

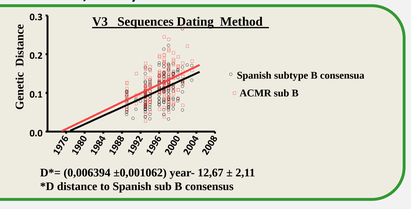
Serial samples from 15 EC, HIV-1 sero-positive individuals without antiretroviral therapy for at least one year and with 2 viral loads determinations < 50 copies/ml were included. After at least 12 months of follow-up, 10 patients showed an increase in viral load > 50 copies/ml (1a) whereas 5 of them maintained the virological control (1b). For virological analysis, we compared viral quasipecies in samples when viral load was <50 copies/ml in both group of patients (blue boxes).



1. Proviral DNA was extracted from PBMC for amplification and sequencing. Viral quasispecies were obtained by PCR limiting dilution in *env* and *gag* genes.



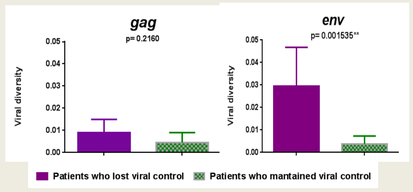
2. Phylogenetic trees were constructed by the Neighbor-Joining method and calculations of viral diversity were performed.
3. Estimation of viral dating was carried out as described (Casado et al., 2000)



4. The presence of escape mutations in the gag gene were also analyzed.

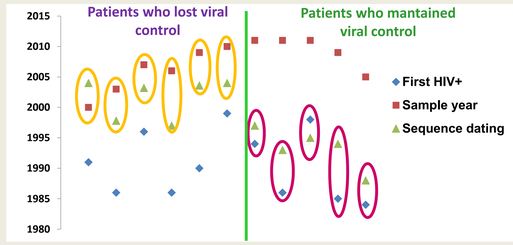
Results

Analysis of viral diversity



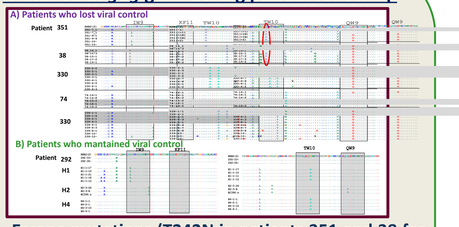
No statistically differences in viral diversity in *gag* gene were observed.
Viral diversity in *env*, in samples when viral load <50 copies/ml was statistically superior (p = 0.004) in patients who lost control (mean = 0.03 ± 0.01) than in those who maintained control (mean = 0.0028 ± 0.003).

Comparison of sample viral dating, first HIV-1 positive and sample year

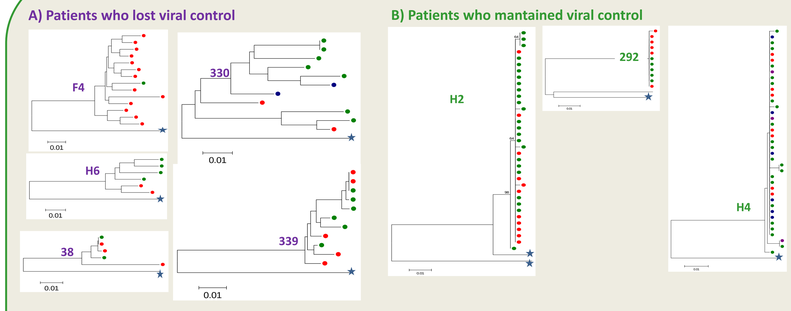


In patients who lost viral control, sample sequence dating ▲ was very close to the sampling date ■ (orange circle).
In contrast, patients who mantained viral control, sample sequence dating ▲ coincided with the date of the first HIV+ date (pink circle).

Mutation in *gag* gene during patient follow-up

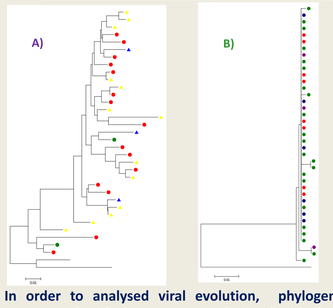


Escape mutations (T242N in patients 351 and 38 for example) were detected in some sequences but these mutations pre-existed in the samples previous to the loss of control (first lines in dark grey). Overall, escape mutations in *gag* could not be related to loss of control.



In spite of undectable level of viral load T=-2 (●) and T=-1 (●), phylogenetic trees showed viral diversity in the patients who lost viral control (A). However, in patients who remained undetectable during the follow up (B), the majority of the sequences were identical in all the samples analysed.

Viral evolution along the follow-up in both group of patients



Two very different patterns of viral evolution were observed between both group of patients. Patients who lost control showed viral diversification before and after the viral lost control (A). Patients who always mantained undetectable viral load (B) did not show evolution during the follow up.

In order to analysed viral evolution, phylogenetic trees obtained by Neighbour Joining were constructed with the sequences obtained with the samples taken in T=-2 (●) and T=-1 (●) when viral load was <50 and with samples taken when patients showed an increase of viral load T=0 (▲) and T= +1 (▲) One representative patient of those who lost viral control (A) and patients who mantained viral control (B) in T=-2 (●) and T=-1 (●), T= 0 (●) and T=+1 (●) are shown.

CONCLUSIONS

- ✓ EC patients with virological control showed no signs of evolution (evolutionary stasis), suggesting that the virus has not replicated since primo-infection.
- ✓ In EC patients who lost viral control, we detected viral diversification even in the samples with undetectable viral load. This indicates that viral replication occurred along the infection.
- ✓ Thus, lack of viral evolution could be used as a diagnostic marker for the identification of true EC. These EC patients could be considered as potentially cured.