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LACK OF TEMPORAL STRUCTURE FOR THE SHORT-TERM VIRAL EVOLUTION IN HIV-1 ASYMPTOMATIC NAIVE PATIENTS

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ABSTRACT

We performed a longitudinal analysis of viral evolution at the C2-V5 region in the HIV-1 env gene in six ART-naïve patients. All patients exhibited a good control of viral replication and no symptoms of clinical progression after 7 to 20 years of infection. Between three to six time points per person were analyzed, covering from two to five years of infection at variable time points since seroconversion. This study demonstrates the existence of at least two different patterns of short-term HIV-1 evolution. One pattern was characterized by the existence of a temporal structure similar to that previously described by Mullins and co-workers (1), with a linear increase in divergence and heterogeneity of the viral quasispecies over time, driven mainly by non-synonymous mutations. The other pattern, not yet described in untreated patients, was characterized by a lack of temporal structure with random fluctuations in the viral divergence and heterogeneity of the quasispecies, principally driven by synonymous mutations. In some patients, the detection of a temporal structure was affected by the stochastic emergence of highly divergent variants that could have their origin in other viral compartments or latent virus reservoirs.

1- INTRODUCTION

During the asymptomatic period of HIV-1 infection, the rapid and continuous viral turnover together with the highly error prone mechanism of virus replication lead to the generation of multiple distinct variants best described as viral quasispecies. A consistent model of evolution over time for HIV-1 quasispecies was proposed by Shankarappa and coworkers, based on the analysis of nine patients with a typical clinical disease progression (1). However, it is not clear whether this pattern of viral evolution can be generalized to HIV-1 quasispecies from patients with other disease progression rates.

2- METHODS

In this study, we performed a longitudinal analysis on viral evolution at the C2-V5 region of the HIV-1 *env* gene in six HIV-1-infected individuals, 4 homosexual (patients I to IV) and 2 intravenous drug users (patients V y VI) from an outpatient clinic (Centro Sanitario Sandoval, IMSALUD, Madrid). Patients were selected on the basis of the their low plasma RNA viral load (usually below 10,000 copies/ml in plasma), CD+ T cell count of more than 500 cells/µl and an asymptomatic state for more than seven years of infection without antiretroviral therapy. Between three to six time points per person were analyzed, covering two to five years of infection at variable time points since seroconversion date (Figure 1).

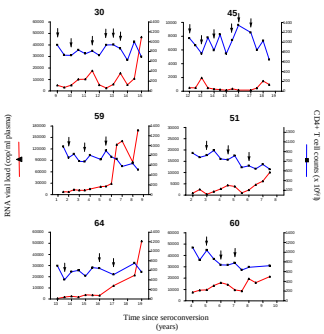

Figure 1. Plasma RNA viral load and CD4+ T cell values over time since the first visit in each of the six patients studied. For samples with a RNA viral load below the detection limit (50 cp/ml), we assigned a value of 50 cp/ml. The arrows indicate the samples in which the viral quasispecies were analyzed. Subject identification numbers are given in the upper central part of each graph.

3.1- VIRAL EVOLUTION WITH A TEMPORAL STRUCTURE

The variants isolated at earlier time points in the viral quasispecies gave rise to the later variants which were more distant to the MRCA than the first ones (see Figure 2A).

• Linear increase in both heterogeneity and divergence of the viral population were observed over time (see Figure 2B).

• Constant increase of the *dN* values (*slope* = 0.011; *P*<0.05) which accumulate at a higher rate over time than *dS* (*slope* = 0.003; *P*=0.12), and the ratio *dN/dS* at each time point was usually higher than one (see Figure 2C).

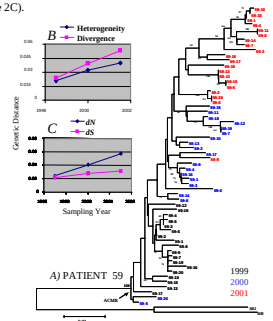

Figure 2. *A* Neighbor-Joining tree of HIV-1 PBMC-derived C2-C5 gp120 *env* sequences (positions 7068 to 7682) from patient 59, along with reference strains (*italic*) as outgroups). Horizontal branch lengths are draw to scale. The arrows identify the position in the phylogenetic tree of the MRCA sequence for each patient. *B* Heterogeneity (mean nucleotide distance between all pairwise comparisons of sequences from within each sample) and Divergence (mean nucleotide distance between all sequences from each sample and the patient MRCA sequence) of the viral quasispecie over time. *C* Accumulation of *dN* (mean non-synonymous substitution per potential non-synonymous site between all sequences from each time point and the MRCA sequence) and *dS* (mean synonymous substitution per potential synonymous site between all sequences from each time point and the MRCA sequence) in the viral quasispecie over time.

3.2- EVOLUTION WITHOUT A TEMPORAL STRUCTURE

The variants that circulated at a given time point were not the origin of the virus isolated immediately later, and more importantly the viruses isolated at later time points were not more distant from the MRCA than viruses isolated earlier (see Figure 3A and D).

• The heterogeneity and the divergence fluctuated in a random manner over time (see figure 3B and E).

• No consistent temporal trends in the patterns of *dN* accumulation were observed, and the *dN* values were generally equally or lower than the *dS* values (see Figure 3C and F).

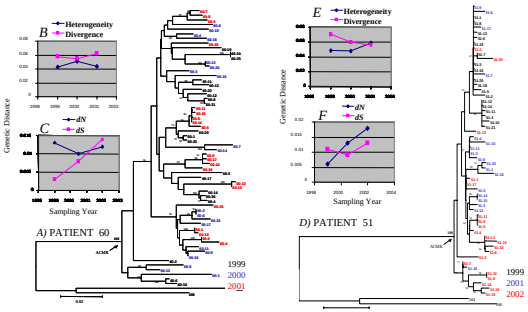

Figure 3. Neighbor-Joining trees of HIV-1 PBMC-derived C2-C5 gp120 *env* sequences (positions 7068 to 7682) from patients 60 (*A*) and 51 (*D*) , along with reference strains (*italic*) as outgroups. As explained in Figure 1. Heterogeneity and Divergence of the viral quasispecies of patient 60 (*B*) and 51 (*E*). As explained in Figure 1. *C* and *F* Accumulation of *dN* and *dS* in the viral quasispecies of patients 60 and 51. As explained in Figure 1.

3.3- PRESENCE OF MULTIPLES SEQUENCES CLADES

In some patients the HIV-1 evolution was characterized by the presence of highly divergent sequences clades within the viral quasispecies (see Figure 4).

• The major clade "I" within each patient display an evolutionary pattern with (patients 30 and 64) or without (patient 45) temporal structure similar to those previously described (see Figure 5A).

• However, these patterns were not clear when some minor clades of the quasispecies were included into the analysis. In particular, clades IV and V in patient 30, clade II in patient 64, and clades II to IV in patient 45, reduce the divergence and increase the mean heterogeneity of the quasispecies (see Figure 5B).

• These genetics changes, are compatible with the hypothesis that these minor clades could have their origin in some latent viral reservoirs (2).

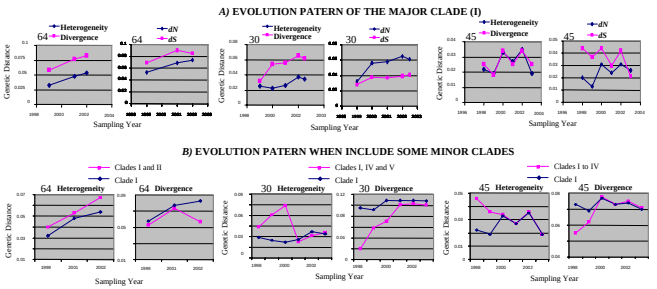

Figure 5. *A)* Heterogeneity, divergence and accumulation of *dN* and *dS* over time, in the major clade I of the viral quasispecies from patients 30, 45 and 64. Heterogeneity, divergence and *dN* and *dS* values were estimated as explained in Figure 1. Subject identification numbers are given in the upper left corner of each graph. *B)* Effect of the introduction of other sequences clades on the heterogeneity and divergence estimations, respect to those defined for clade I. Subject identification numbers are given in the upper left corner of each graph.

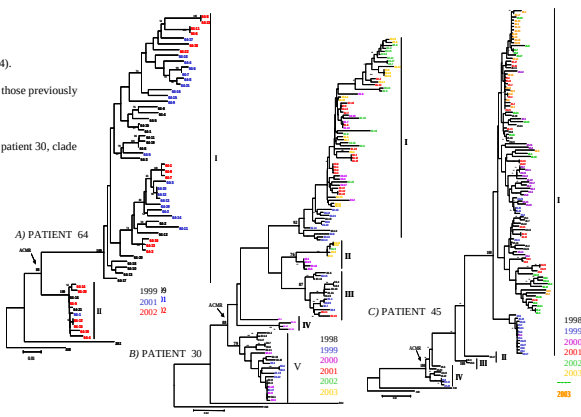

Figure 4. Neighbor-Joining trees of HIV-1 PBMC-derived C2-C5 gp120 *env* sequences (positions 7068 to 7682) from patients 64 (*A*), 30 (*B*) and 45 (*C*) , along with reference strains (*italic*) as outgroups. As explained in Figure 1. Nucleotide sequence clusters within each patient are grouped by vertical lines and denominated by numbers I to V.

4- CONCLUSIONS

In this study we detected two patterns of short-term viral evolution in HIV-1 asymtomatic patients:

• One pattern displays a temporal structure dominated by positive selection.

This pattern is similar to the model proposed for typical progressors by Shankarappa and coworkers (1).

• The other pattern do not show a temporal structure and is driven by genetic drift.

This pattern concur with the PAT model (3) and has been only previously described for reservoir sequences from patients under HAART (4,5).

References:

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