

SINGLE GENOME SEQUENCE ANALYSIS OF PLASMA VIRUSES FROM HIV-1 CONTROLLERS SUGGESTS ONGOING LOW-LEVEL REPLICATION

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Background: HIV-1 controllers are infected individuals who, without therapy, achieve HIV-1 RNA levels below the limit of detection measured using standard polymerase chain reaction assays (<50 copies/ml). Although these patients have been reported to have low levels of viremia measured using single copy amplification assays (SCA), the underlying replication dynamics remain poorly understood. In this study single genome sequencing (SGS) was used to characterize sequence diversity and divergence of *pro* and *pol* in longitudinal sampled plasma samples from HIV-1 controllers compared to non-controllers.

Methods: Study subjects included 21 HIV-1 controllers (HIV-1 RNA <50 copies/ml for at least 90% of measurements for a minimum of 4 years) and 40 HIV-1 non-controllers (HIV-1 RNA >10,000 copies/ml at all time points for a minimum of 4 years). HIV-1 RNA was extracted from 3-6 ml of plasma and assayed by single-copy assay (SCA) and single genome sequencing (SGS). Phylogenetic trees were constructed in MEGA 4.1 using neighbour joining (NJ) algorithms. Genetic diversity was estimated as average pairwise distances (APD) and sequence divergence was assessed using the Nei and Gojobori method.

Results: Median HIV-1 RNA in 36 samples from HIV-1 controllers was <0.7 copies/ml (range <0.4-20). A total of 199 *pro-pol* sequences was obtained by SGS from 13 of 21 controllers (sequences derived from 69 of 170 tested plasma samples). Intra-patient NJ trees showed time-dependant clustering of sequences supported by bootstrap values >80 and increasing divergence with time indicating evolution of the viral population. Median sequence diversity was significantly lower in HIV-1 controllers (median 0.4%; range 0-1.9%) than in non-controllers (0.8%; range 0.13-3.15% [Mann Whitney p=0.0003]). Sequence diversity increased slowly with time or was unchanged in HIV-1 controllers ($r^2=0.01$, $p=0.58$), whereas diversity in non-controllers increased significantly over a similar time interval ($r^2=0.14$, $p=0.0009$). Estimates of dN/dS were <1 in both controllers and non-controllers suggesting ongoing purifying selection in both patient groups.

Conclusions: HIV-1 controllers have intermittent low-level viremia. Time dependent clustering of plasma sequences and increasing divergence suggests ongoing viral replication, although diversification of the viral population in HIV-1 controllers is restricted and significantly lower than that in HIV-1 non-controllers.