Longitudinal analysis of infection frequencies and genetic makeup of intracellular HIV-1 from tissue compartments during long-term suppressive therapy

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Background: Efforts to eradicate HIV-1 require a comprehensive examination of the quantity and genetic makeup of HIV-1 populations within infected cells located in tissues throughout the body. Therefore, we conducted a longitudinal analysis of the infection frequencies and genetic makeup of HIV-1 in specific CD4+ T-cell subsets in different tissue compartments from patients on long-term suppressive therapy.

Methods: Using single-genome and single-proviral sequencing techniques, we isolated intracellular HIV-1 genomes derived from defined subsets of T-cells (naïve, central-, transitional-, and effector-memory) from peripheral blood, GALT, and lymph node tissue. Samples were collected at 2 time points (separated by 6 months) from 8 subjects on suppressive therapy (4-12 years): 5 who initiated therapy during acute infection and 3 who initiated therapy during chronic infection. Maximum likelihood phylogenetic trees were constructed using the general time reversible model.

Results: Comparison of the infection frequencies between the 2 time points showed similar (< 5-fold difference) infection rates of memory T-cell subsets from different tissue compartments for most subjects. However, one subject had a 16-fold increase in the infection frequency of peripheral blood effector-memory T-cells at time point 2. Phylogenetic analyses revealed an increase in clonal DNA sequences with no evidence of genetic evolution in this subject. In agreement with findings for time point 1, infection frequencies of all T-cell subsets were higher in subjects treated during chronic infection than acute infection; time point 2 included transitional-memory T-cells which were not examined at time point 1 (6-fold higher infection rate in chronic vs acute; p=0.036). Approximately 30% of the intracellular HIV sequences encoded replication-incompetent virus. In one subject, a clonal species containing a 380bp deletion was dominant, and increased from 71% to 92% over 6 months in peripheral blood effector-memory T-cells.

Conclusions: Our findings suggest the pool of HIV-infected resting memory CD4+ T-cells typically does not change dramatically over 6 months in different tissue compartments, reflecting a relatively stable HIV-infection frequency during suppressive therapy. The increase of clonal HIV-1 sequences from effector-memory T-cells in 2 subjects, especially a large deletion mutant, indicates an expansion of cells with integrated proviral DNA rather than active viral replication.