

ANALYSIS OF HIV DNA MOLECULES IN SINGLE INFECTED CELLS FROM RECENTLY AND CHRONICALLY INFECTED PATIENTS

L. Josefsson¹, J. Brännström², B. Makitalo², F. Maldarelli³, M. Kearney³, W. Shao³, D. Rock⁴, J. Albert¹, J. Coffin⁵, and S. Palmer^{1,3}

¹Department of Virology, Swedish Institute for Infectious Disease Control, Karolinska Institute, Solna, Sweden; ²Department of Immunology, Swedish Institute for Infectious Disease Control, Solna, Sweden; ³HIV Drug Resistance Program, NCI, NIH, Frederick, MD; ⁴NIAID/CCMD Clinic, NIH, Bethesda, MD; ⁵Tufts University, Boston, MA

Background: The number of HIV-1 proviruses within individual infected cells in HIV-infected patients has not been well defined. Furthermore, the genetic relatedness of proviruses within infected cells and between cells and plasma virus is unknown. To address these issues we developed techniques to quantify and genetically characterize viral DNA from single infected cells *in vivo*.

Methods: CD4+ T-cells and monocytes were sorted into PCR plates such that each well contained, on average, far less than one infected cell. The cells in each well were lysed and their DNA distributed over a row of 10 wells. A 1.3 kb gag-pol fragment was amplified and sequenced. The number of viral DNA molecules per infected cell was estimated from the number of positive wells in each row, and the relatedness of viral DNA sequences to one another, to DNA in other cells, and to contemporaneous plasma virus RNA was determined.

Results: Analysis of cells from five recently (< 6 months) and four chronically (1-15 years) infected patients with plasma viral RNA levels from 510 to 1,800,000 copies/ml revealed HIV DNA copy numbers varying from one per 93 to one per 2300 CD4+ T-cells. The majority (80-90%) of infected CD4+ T-cells contained a single viral DNA molecule and the number of wells yielding more than one copy of viral DNA was similar to that predicted by the Poisson distribution. Sequence analysis revealed that intracellular viral DNA from CD4+ T-cells in both recently and chronically infected patients was indistinguishable phylogenetically from sequences derived from contemporaneous plasma RNA. The average genetic diversity of the HIV populations from CD4+ T-cells and plasma was 0.4% and 0.2%, respectively. Analysis of 2000-28,000 CD14+ and 73,000-200,000 CD16+ monocytes revealed no HIV-infected cells.

Conclusions: These results suggest that most infected CD4+ T-cells in blood contain only one copy of HIV DNA, implying a limited potential for recombination in virus produced by these cells. The infection rate of CD4+ T-cells is at least 30-fold greater than CD16+ monocytes. The genetic similarity between HIV populations in CD4+ T-cells and plasma implies ongoing exchange between these compartments both early and late after infection.