84

High Levels of Plasma Virus Detected During Asymptomatic, as well as During Acute and Late Stages of HIV-1 Infection By Quantitative Competitive PCR (QC-PCR), M. Piatak, Jr.[1], M.S. Saag[2], L.-M. Yang[1], S.J. Clark[2], J.C. Kappes[2], K.-C. Luk[1], B.H. Hahn[2], G.M. Shaw[2], and J.D. Lifson[1], [1]Genelabs Incorporated, Redwood City, CA, USA 94063 and [2]The University of Alabama at Birmingham, Birmingham, AL, USA 35294

The ability to monitor virus load accurately throughout all stages of HIV-1 infection, particularly during the asymptomatic period when patient plasma is typically negative for viral antigen and infectious virus, would provide a critically needed non-clinical endpoint for the evaluation of disease status and the efficacy of therapeutic intervention. Standard PCR methods offer the extreme sensitivity required for detection of low levels of virus but may not reliably provide accurate quantitation due to varying efficiencies of amplification associated with different input amounts of target sequence and sample composition. Competitive PCR methods obviate these problems and afford the most reliable means of quantitating specific nucleic acid species. We have applied Quantitative Competitive PCR (QC-PCR) methods, together with sample processing methods designed to maximize nucleic acid recovery, to the evaluation of plasma specimens from 66 infected subjects with different stages of disease. Significantly, HIV-1 RNA was detected and quantitated in plasma at all time points from all subjects but one, representing all stages of disease. The results provide evidence for ongoing viral replication throughout infection. Determined values ranged from 2,000 to more than 20,000,000 RNA copies per ml of plasma. These levels are 10 to 100-fold higher than have been reported previously in similar stage patients using other PCR methods. The highest levels of plasma virus RNA were detected in early, acute infection and in CDC Stage IV disease. The QC-PCR determined virus levels correlated with disease stage and with absolute counts of CD4 + T-cells. The determined virus levels also correlated with, but exceeded by 1,000 to 10,000-fold, titers of infectious units determined by endpoint dilution culture. These results demonstrate that the level of viremia throughout all stages of HIV-1 disease is much higher than thought previously and suggest that both non-infectious and infectious forms of virus may contribute to pathogenesis. QC-PCR methods offer a reliable means of quantitating HIV-1 in clinical and other specimens and may be useful in following the course of infection and the effects of therapeutic interventions. Similar methods should be useful in studying other infectious agents, particularly in specimens in which the levels of virus replication or expression are below the limits of more traditional methods of detection.

85

Direct Evidence Using QC-PCR Methods for Persistent, High Levels of Circulating Virus in Acute and Early-Chronic HIV-1 Infection, M. Piatak, Jr.[1], L.-M. Yang[1], S.J. Clark[2], J.C. Kappes[2], K.-C. Luk[1], B.H. Hahn[2], M.S. Saag[2], J.D. Lifson[1], and G. Shaw[2], [1]Genelabs Incorporated, Redwood City, CA, USA 94063 and [2]The University of Alabama at Birmingham, Birmingham, AL, USA 35294.

Acute symptomatic infection by HIV-1 (CDC Stage I) is characterized by clinical signs of immune activation, multi-system organ dysfunction, and high peak levels of viremia, p24 antigenemia and proviral burden. In association with seroconversion, clinical symptoms typically resolve and circulating infectious virus and antigen levels decline precipitously to undetectable or barely detectable levels in most patients. We have applied highly sensitive and accurate Quantitative Competitive PCR (QC-PCR) methods, in addition to standard assessments of infectious virus titers, p24 antigenemia, and CD4 + T-cell levels, to the evaluation of six patients presenting with acute symptomatic HIV-1 infection and followed for 1-3 years. In these patients, infectious virus in plasma (10-10,000 TCID/ml) and p24 antigen (306-5,405 pg/ml) peaked within 14 days of symptom onset. Within 115 days following presentation, infectious virus and p24 antigen levels were undetectable or minimally positive and continued to be detected at only slightly higher levels, if at all, through the course of the study. In contrast to all other virologic markers assayed, QC-PCR determined virus RNA levels were readily quantified in all patients at all time points. The profile of virion associated RNA levels generally paralleled the other virologic markers, showing peak values between 3.55×10^6 and 2.2×10^7 from 8 to 23 days after presentation. Over the period of follow-up, RNA levels fell by up to 650-fold from peak levels, persisting in the range of 2×10^3 to 3.3×10^5. Among the six patients there were apparent correlations between the profiles of virus RNA load and trends in CD4 + T-cell counts and clinical status. The absolute levels of circulating virus in conjunction with primary HIV-1 infection thus may be one important correlate of subsequent depletion of CD4 + cells and clinical outcome, although other factors, including viral phenotype and host immune response, are also likely to be involved. QC-PCR shows promise as a method for sensitively and accurately assessing viral load at all stages of infection. Further studies will help to define the role of total virus levels in the pathogenesis of HIV-1 infection and the utility of this parameter as a prognostic indicator.