Low-incidence latent infection with variant B or roseola type human herpesvirus 6 in leukocytes of healthy adults

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Summary. Nested primer-based polymerase chain reaction was employed to determine the frequency of latent infection with human herpesvirus 6 (HHV-6) among healthy adults from Bratislava, Slovak Republic. A 592-bp region, upstream from the gene encoding the putative large tegument protein of HHV-6, was amplified from DNA extracted from peripheral blood mononuclear cells (PBMC) of only one of 29 seropositive adults, suggesting that as few as 1 in $10^5$ PBMC may be infected with the virus. Direct sequencing of the 592-bp fragment indicated that the virus harbored by the seropositive Slovak subject (designated B38) differed by only 3 nucleotides from an HHV-6 variant B strain (R-147) isolated from an American infant with a roseola-like illness and by 32 bases from the variant A strain GS isolated from a patient with lymphadenopathy (5.4% sequence divergence). None of these strains had a deoxyadenosine at base position 1251, when compared to the published sequence of strain GS clone pZVH14. Although this discrepancy did not affect the large tegument protein gene, it altered the predicted amino acid sequences of two putative proteins coded by open-reading frames 1 and 2 (ORF 1 and ORF 2) located upstream from this gene.

Introduction

Human herpesvirus 6 (HHV-6), originally isolated from cultures of peripheral blood mononuclear cells (PBMC) derived from patients with lymphoproliferative disorders [30] and acquired immunodeficiency syndrome (AIDS) [22, 27, 38], is now known to be the etiological agent of roseola infantum or
exanthem subitum [39] and possibly an infectious mononucleosis-like syndrome [3, 19, 26, 34]. HHV-6 infection is usually acquired during infancy and childhood, and infection is widespread in populations from widely separated geographical regions [5, 6, 20, 27]. Although HHV-6 was initially believed to be B-cell lymphotropic, accumulated data indicate that mononuclear cells which support HHV-6 replication display markers characteristic of T cells, namely CD2, CD4, CD5, CD7, and CD8 [23, 35], but not markers of B cells, such as CD21 or CD20 [9].

Like other human herpesviruses, HHV-6 can establish latent infection with nonintegrated viral DNA persisting in a covert, nonproductive form. Kondo and colleagues [18] demonstrated that adherent monocytes harbor HHV-6 for more than a month without expression of detectable antigen or virus replication. Similarly, HHV-6 genomic sequences were detected in monocytes by polymerase chain reaction (PCR), without detectable viral antigen, during the convalescent phase of roseola [16, 17]. PCR has also been employed to demonstrate HHV-6 DNA in throat washings and leukocytes of a small proportion of healthy adults [15] and patients infected with human immunodeficiency virus (HIV) [31] and in lymph nodes of some patients with Hodgkin’s and non-Hodgkin’s lymphomas [11, 37]. Here, we report the enzymatic amplification and direct sequencing of a 592-base pair (bp) region of HHV-6 in DNA extracted from PBMC of a healthy adult from the Slovak Republic. Our analysis also indicates a possible error in the originally reported sequence of HHV-6 strain GS. This finding alters the predicted amino acid sequences of the two proteins encoded upstream to the tegument gene and markedly increases the predicted size of the protein encoded by open-reading frame (ORF 1).

Materials and methods

Blood specimens and DNA extraction

Heparinized blood (5-10 ml) was obtained with informed consent from 45 healthy adults (age range, 20-60 years) in Bratislava, Slovak Republic. Plasma was saved and PBMC were separated by Ficoll-gradient centrifugation. DNA was extracted from uncultured PBMC by the saturated phenol-chloroform method following proteinase K digestion. From suspensions of HHV-6 infected and uninfected HSB-2 cells (10^7 cells/ml), DNA was extracted into PCR buffer, as described by Higuchi [10].

Viruses and cells

Uninfected HSB-2 cells and HSB-2 cells infected with the GS strain of HHV-6 variant A were provided by the AIDS Research and Reference Reagent Program, Rockville, MD, U.S.A. Uninfected J-Jhan cells and J-Jhan cells infected with the R-147 strain of HHV-6, isolated from a 5-month old female infant with a roseola-like illness characterized by high fever (40.5 °C), malaise, bulging anterior fontanelle, diarrhea and rhinorrhea but without rash, were kindly provided by Dr. C. B. Hall, Dr. M. T. Caserta and Ms. K. McIntyre of the Department of Pediatrics, University of Rochester, School of Medicine, Rochester, NY, U.S.A. Cells were maintained in culture as previously described [39].