Human Herpesvirus-6 Revisited

Since the initial 1986 isolation of the human herpesvirus-6 (HHV-6), from the peripheral blood mononuclear cells (PMNCs) of patients with acquired immunodeficiency syndrome (AIDS) and lymphoproliferative disorders, there has been considerable progress in characterizing its morphology, immunologic and growth properties, and genetic architecture. The virus has a wide cellular tropism infecting both B and T lymphocytes (mainly CD4+ cells, but also CD2, CD5, CD7, and CD8) as well as megakaryocytes and neural cells. Several studies, some using immunoperoxidase and in situ hybridization methods, suggest that the salivary gland is a possible site of HHV-6 replication and persistence. HHV-6, like other herpesviruses, has a 160- to 200-nm enveloped virion that contains an icosahedral nucleocapsid with 162 capsomeres and a large, central double-stranded DNA genome. Nucleotide sequencing studies have shown a high degree of homology between HHV-6 and cytomegalovirus; however, unique HHV-6 DNA sequences allow for specific primers and probes for diagnostic in situ hybridization (ISH). Southern blot analysis, and polymerase chain reaction assays. Isolation of HHV-6 from clinical samples is best accomplished by culture using mitogen-stimulated mononuclear cells from cord blood samples. Infected cell cultures show cytopathic effects characterized by large refractile mono-nucleate or binucleate cells that are frequently accompanied by inclusion bodies and cell lysis. Viral isolates have shown varying degrees of strain heterogeneity. In contrast to Epstein-Barr virus, cellular growth immortalization by complete HHV-6 particles has not been demonstrated.

A variety of immunoassays (indirect fluorescent centibody test, enzyme-linked immunosorbent assay) and neutralization tests are available for detection of IgM and IgG-specific HHV-6 antibodies. Also, Western blotting methods can be used to evaluate the interactions of human sera and purified HHV-6 viral proteins. Antibody prevalence studies suggest that the HHV-6 infection is frequently silent. The high prevalence of IgG antibodies in infants correlates with acute seroconversions as passively acquired antibody wanes. As a result, more than 90% of young children between the ages of 1 and 4 years are anti-HHV-6-positive. Most (81–93%) blood donors and other healthy adults are seropositive for HHV-6 by either enzyme-linked immunosorbent assay or indirect fluorescent antibody test. Although other studies with different test cutoff values show a lower prevalence of HHV-6 seropositivity, the overall picture derived from HHV-6 seroepidemiology suggests that few adults are still susceptible to primary HHV-6 infection. Because a variety of interfering substances, including rheumatoid factors and heterotopic cross-reactions, can interfere in viral-specific IgM assays, IgM/IgG separation by pretreatment of serum with anti-IgG is recommended before a specific IgM HHV-6 assay is performed. Even then the detection of IgM responses only indicates an active viral process rather than implying an ongoing primary infection. The specificity of IgG responses is also problematic. Initially, there was concern about serologic cross-reactions between the cytomegalovirus and HHV-6; however, subsequent serum absorption studies with either HHV-6 or the cytomegalovirus removed only homologous IgG antibody, making cross-reactivity unlikely. Subsequently, it became clear that viruses like the cytomegalovirus and Epstein-Barr virus could reactivate the HHV-6 carrier state, yielding brisk fourfold or greater IgG HHV-6 responses. Because of such HHV-6 carrier state reactions and the possibility of dual simultaneous viral infections, caution has been urged in interpreting HHV-6 data, i.e., the cytomegalovirus and Epstein-Barr virus hyperactivity must be ruled out before accepting a serologic basis that HHV-6 is the cause of a particular illness.

Human herpesvirus-6, once described as a "virus in search of a disease," has now been clearly linked to exanthem subitum (roseola infantum), the short-lived febrile viral illness of infancy, following its isolation from the PMNCs of such infants. The virus was accompanied by HHV-6 seroconversions. The acute-phase isolations were derived from nonadherent CD4+CD8− or CD3+CD4+ cells, but not from CD4−CD8+ or B cells. With more sensitive polymerase chain reaction methods, positive cells were found in both the adherent and nonadherent fractions of PMNCs from infants with acute exanthem subitum. When these studies were repeated with mononuclear cells from convalescence (1.5–2.5 months after onset) and from healthy adult blood donors, HHV-6 was found only by polymerase chain reaction in adherent cells, most of which were nonspecific esterase-positive monocytes. These data raise the question of whether monocytes/macrophages are a site for HHV-6 latency in vivo. Despite strong linkage of HHV-6 to roseola, not all clinical cases are associated with HHV-6 isolations and seroconversions. Also, in another recent hospital emergency room study of young children between the ages of 3 months and 3 years with a variety of febrile illnesses, transient viremia and HHV-6 seroconversions were observed in 13% of cases, only a minority of which were associated with exanthematous rashes.

The association of HHV-6 with entities other than exanthem subitum remains uncertain. Several studies suggest that HHV-6 is capable of causing a non-Epstein-Barr virus/cytomegalovirus heterophil-negative infectious mononucleosis-like illness in previously healthy individuals. Many of these cases have complete serologic data and impressive atypical blood smear findings; however, for the most part they lack accompanying viral isolation studies. Increased HHV-6 antibody prevalence also has been reported in groups of patients with the chronic fatigue syndrome; African (Burkitt's) lymphoma; and some collagen vascular diseases, including Sjögren's syndrome and malignant lymphoma. Some of these data can be attributed to HHV-6 carrier state reactions secondary to impaired cellular immune responses associated with the primary disease processes. Human herpesvirus-6 also has been linked recently by serology and spinal fluid polymerase chain reaction studies to some cases of the Guillain-Barre syndrome and encephalomyelitis.

The virus also has been studied in immunosuppressed organ transplant recipients. In a recent serial study of bone marrow
pressed patients, who frequently have increased viral loads. The virus has also been shown in vitro to be capable of suppressing bone marrow function, and has been causally linked to an occasional case of virus-associated hematopoietic syndrome. Human herpesvirus-6 may play an unspecified role in renal allograft rejection. Finally, the interaction of HHV-6 and human immunodeficiency virus Type I in AIDS states has been studied both in vitro and in vivo with no consensus of opinion regarding the role of HHV-6 in the evolution of HIV.

Because HHV-6 was first isolated from patients with lymphoproliferative disease and is associated with lymphoproptosis in vitro, it seemed natural to search for HHV-6 sequences in diseases associated with lymphadenopathy. This has been accomplished using molecular biology methods, including DNA extraction with Southern blot analysis, the polymerase chain reaction amplification technique, and in situ hybridization studies on tissue sections. The pitfalls and limitations of these techniques have been the subject of several recent publications, including an editorial in this journal. Variables influencing in situ hybridization results include the tissue type and fixation conditions, the size and type of nucleic acid probes, and the stringency of posthybridization washes. Negative controls, including similarly prepared heterologous nucleic acid probes, must be used to assess the specificity of the probe binding. Critical factors for polymerase chain reaction sensitivity include not only the amount of template used but also factors such as the magnesium concentration of the reaction buffer, which must be optimized for each primer pair. Specificity can be achieved by meticulous avoidance of cross-contamination and by using a virus-specific probe that does not contain a primer sequence in the final detection of the polymerase chain reaction product. Conventional polymerase chain reaction studies are usually insufficient to differentiate viral latency from active HHV-6 infection. Another practical problem deals with interpretation of the above test data in immunosuppressed patients, who frequently have increased viral loads.

In a search for HHV-6 DNA in biopsy specimens from patients with malignant lymphoma and Hodgkin's disease, the polymerase chain reaction is usually used for screening purposes, and is followed by confirmation with Southern blot analysis and localization with in situ hybridization. Most studies suggest that HHV-6 genomes are not commonly found in such tumors and normal control tissue. In one such study using Southern blot analysis, mainly negative data were found in more than 100 tissue biopsies from patients with non-Hodgkin's lymphoma (3/104), Hodgkin's disease (0/8), reactive lymphadenitis (0/31), and other neoplastic conditions (0/22). Krueger and colleagues studied HHV-6 in atypical polyclonal lymphoproliferative disorders and malignant lymphoma, and concluded that the virus was etiologically involved in a small percentage of cases. They theorized that HHV-6 could stimulate polyclonal B-cell activation and lower the oncogenic threshold by expanding the population targeted by independent transforming events.

Recently the role of HHV-6 in sinus histiocytosis or Rosai-Dorfman disease was evaluated in a well-controlled study using paraffin blocks and in situ hybridization methods. When tissue from sinus histiocytosis was tested, HHV-6 sequences were found in seven of nine cases. Genomes of Epstein-Barr virus alone were found in one other case, and neither HHV-6 nor Epstein-Barr virus was detected in the last case. Most HHV-6 staining was found in the abnormal cells of the sinusoids that stained as histiocytes by immunoperoxidase methods. When this technique was used on more than 100 different biopsy specimens taken from patients with Burkitt's lymphoma, Hodgkin's disease, malignant lymphoma, and nonspecific lymphadenitis, HHV-6 sequences were seldom identified. These authors discussed their data in relation to a pathogenic role for HHV-6 in sinus histiocytosis (Rosai-Dorfman disease).

In an article in this issue of the American Journal of Clinical Pathology, Sumiyoshi and associates have used many of the techniques described above to evaluate HHV-6 in histiocytic necrotizing lymphadenitis (or Kikuchi's disease). This study follows previous reports on histiocytic necrotizing lymphadenitis from the authors' laboratories in which the clinicopathologic features, immunohistochemical and HLA findings, and other data were reported. In the present study of cervical lymph nodes, positive HHV-6 polymerase chain reaction data were recorded in all cases of histiocytic necrotizing lymphadenitis. Accompanying in situ hybridization and Southern blot analysis studies were mainly negative. The significance of these findings is not entirely clear; however, their data must be appraised in light of control data presented. Points of concern include the absence of reactivity of two of their primer pairs; the number of positive polymerase chain reaction tests in control lymph nodes, including some from patients with tuberculosis; and the absence of positive supportive in situ hybridization or Southern blot analysis data on tissues from histiocytosis necrotizing lymphadenitis. Further evaluations are indicated before accepting HHV-6 as an agent capable of triggering histiocytic necrotizing lymphadenitis.

Much has been learned about this new member of the herpesvirus family since it was discovered 7 years ago. Its association with exanthem subitum (roseola) seems secure. If this virus lives up to its family name, however, it will have a complex life cycle leading to a variety of clinical manifestations. Tantalizing pieces of evidence suggest possible associations with lymphoproliferative disorders in adults and disease in immunocompromised patients. Because of the high prevalence of HHV-6 infection, further understanding of its pathogenesis would be aided by tissue studies designed to distinguish presumed latent from active infection. In this regard, new sensitive molecular techniques, such as those combining polymerase chain reaction and in situ hybridization, may enhance detection of latent virus, clarifying the molecular biology of HHV-6 and opening new avenues for investigation.

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References


