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Human Herpesvirus 6

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INTRODUCTION

Humans are the primary hosts for eight known herpesviruses. Herpes simplex virus type 1 (HSV-1) (human herpesvirus 1) has infected about 70% of adults and is the etiologic agent of common fever blisters; it occasionally causes severe encephalitis. Herpes simplex virus type 2 (HSV-2) (human herpesvirus 2) has infected about 30% of adults and is commonly the cause of genital herpes; it occasionally causes severe infections in neonates. Varicella-zoster virus (VZV) (human herpesvirus 3) has infected more than 90% of adults and causes varicella (chicken pox) and herpes zoster (shingles). Over 90% of adults have been infected with Epstein-Barr virus (EBV) (human herpesvirus 4), the cause of about 85% of infectious mononucleosis cases. EBV is also associated with Burkitt's lymphoma and nasopharyngeal carcinoma. Human cytomegalovirus (HCMV) (human herpesvirus 5) has infected about 70% of adults and is the cause of 10 to 15% of infectious mononucleosis cases. Congenital infection with HCMV is a major cause of deafness and mental retardation. The clinical spectrum of human herpesvirus 7 (HHV-7) has not been completely defined, but primary HHV-7 infection has been associated with some cases of exanthem subitum (ES) and other exanthems clinically similar to measles or rubella. Human herpesvirus 8 (HHV-8) was recently discovered in patients with AIDS-associated Kaposi's sarcoma (KS) lesions, and HHV-8 infection has been associated with other forms of KS, as well as other proliferative diseases. Table 1 provides a summary of some of the pathogenic and epidemiologic properties of the human herpesviruses.

The entity known as human herpesvirus 6 (HHV-6) consists of two closely related yet distinct viruses, designated human herpesvirus 6 variant A (HHV-6A) and human herpesvirus 6 variant B (HHV-6B). Current serologic methods cannot readily discriminate prior infection with one virus from prior infection with the other. Over 95% of people older than 2 years of age are seropositive for either or both viruses. HHV-6A has not been etiologically linked to any human disease, but such an association will probably be found in the near future. HHV-6B is the etiologic agent of the common childhood illness ES (also known as roseola infantum or sixth disease) and related febrile illnesses and frequently is active in immunocompromised patients.

In this review, we will describe what is known of the molecular and cellular biology of HHV-6A and HHV-6B, as well as their biology in their host organism, including their role in human disease. Thus far, nearly everything published on the subject of these viruses has described them both as HHV-6. It is possible to reinterpret many earlier reports in the light of the new grouping. To the extent possible, we will treat the viruses as separate entities.

HISTORICAL AND CLINICAL OVERVIEW

This section is intended to provide a concise overview of the historical and clinical aspects of HHV-6 infections. Subjects that are discussed in greater detail elsewhere in this review are fully referenced there.

The discovery of HHV-6A and HHV-6B can be attributed to the development of methods enabling primary lymphocytes to be cultured for extended periods and to the widespread use of these techniques in the study of human immunodeficiency viruses (HIVs). Salahuddin and coworkers first reported the isolation and characterization of a novel herpesvirus from people with AIDS and lymphoproliferative diseases (252, 449, 450). The virus differed from the previously characterized herpesviruses with respect to growth properties, antigenicity, and genetic content. On the basis of an observed tropism for B cells, the virus was named human B-lymphotropic herpesvirus.

The discovery of a novel herpesvirus was quickly confirmed

TABLE 1. Pathogenic profiles of the HHVs[a]

Virus	Subfamily	Age at primary infection	Prevalence in adults (%)	Disease association for:				Cell association during:	
				Primary infection	Reactivation	Chronic infection	Immunocompromised patients	Primary infection	Latency or persistence
HSV-1	α	Most by 20 yr	~70	5/6 subclinical, 1/6 oral lesions, neonatal infections	Oral lesions, encephalitis	Minimal	Large, slow-to-heal lesions	Skin epithelial cells	Neurons of trigeminal ganglia
HSV-2	α	After sexual activity	~30	5/6 subclinical, 1/6 genital lesions	Genital lesions, encephalitis	Minimal	Large, slow-to-heal lesions	Skin epithelial cells	Neurons of sacral ganglion
VZV	α	6 mo to adolescence	>95	Varicella	Herpes zoster	Minimal	Severe herpes zoster	Skin epithelial cells	Cells of trigeminal and dorsal root ganglia
HCMV	β	Most by 20 yr	~70	5% of infectious mononucleosis, congenital CMV	?	Atherosclerosis (?), restinosis (?)	CMV disease, retinitis, gastrointestinal disease	Epithelial cells in a variety of tissues	Monocytes or granulocyte-macrophage progenitors (?)
EBV	γ	Most by 20 yr	~85	>85% of infectious mononucleosis	?	Nasopharyngeal carcinoma, Burkitt's lymphoma	B-cell lymphomas, oral leukoplakia	Oropharyngeal epithelium B cells	B cells
HHV-6A	β	?	>95% for HHV-6A plus	?	?	MS (?)	Disseminated infections, pneumonitis (?), as for HHV-6A	?	Skin, lymphocytes
HHV-6B	β	Before 2 yr	HHV-6B	ES in 30% of children	?	MS (?)		$CD4^+$ lymphocytes	Macrophages, lymphocytes
HHV-7	β	>50% by 2 yr	>85%	subset of ES	?	?	CMV disease	$CD4^+$ lymphocytes	Infectious virus is in saliva
HHV-8	γ	?	?	?	?	KS, multicentric Castleman's disease, primary effusion lymphomas	KS, multicentric Castleman's disease, primary effusion lymphomas	?	sperm?, lymphocytes?

[a] For recent reviews on all the viruses except for HHV-8, see the relevant chapters in reference 163. HHV-8 is reviewed in reference 386.

by several laboratories, and isolates were obtained from specimens collected on several continents, indicating that infection with the virus is widespread (11, 49, 144, 324, 504, 556). Results from several of these laboratories indicated that the newly described virus was most likely to be found and to grow in $CD4^+$ lymphocytes (49, 144, 324, 343, 497, 504). The name of the virus was changed to human herpesvirus 6 (HHV-6), a name independent of the cell tropism of the virus and in accordance with guidelines established by the International Committee on Taxonomy of Viruses (6).

As the cellular and molecular biologic properties of independent isolates of HHV-6 were compared, the segregation of isolates into two groups became apparent. The groups differ with respect to epidemiology (3, 34, 35, 61, 128, 131, 139, 178, 254, 405, 455, 551), growth properties (3, 89, 128, 545), reactivity with panels of monoclonal antibodies (MAb) (3, 34, 89, 128, 455, 545), restriction endonuclease profiles (3, 34, 35, 180, 196, 250, 455), and nucleotide sequences (34, 35, 100, 101, 196, 197, 200, 505, 554). Nonetheless, they are very closely related, with some genes being over 95% identical. After considerable debate in the literature and at international scientific meetings, a decision was made to formally recognize the differences as sufficient to define the two groups as HHV-6 variants A and B (2). HHV-6A is exemplified by strains GS and U1102, and HHV-6B is exemplified by strains Z29 and HST. The presence of the two groups raises several questions. What are the differences between the viruses with regard to disease spectrum and epidemiology? Can a variant-specific serologic test be developed? Can intertypic recombinants be formed? Answers to these and many related questions should be forthcoming in the next few years.

The early literature describing HHV-6 serology shows several inconsistencies. In some reports, as few as 3% of people were described as seropositive for the virus, and in others over 90% were seropositive. Age-related increases as well as declines in seropositivity were noted. Correlations were and were not seen between HHV-6 antibody status and the progression of HIV-related disease. Much of this confusion can be attributed to the lack of sensitivity of some of the earliest serologic tests, as well as to the use of very conservative cutoffs to avoid nonspecific results. Current methods indicate that more than 95% people over 2 years of age are seropositive for either HHV-6A or HHV-6B or both.

The search for diseases associated with HHV-6 infections has been complicated by the high viral seroprevalence, but the early age of acquisition of infection noted by some workers suggested that the virus might be associated with a common childhood disease. Yamanishi et al. found that HHV-6B could be cultured during the febrile phase of the common childhood illness ES (roseola infantum) and, further, that the children demonstrated seroconversion to the virus within weeks of the acute illness (556). Primary HHV-6B infection normally produces mild or even subclinical disease, but more severe presentations have been observed. As described in detail below, these include hepatitis, hemophagocytic syndrome, fatal disseminated infection, and neurologic manifestations (e.g., febrile convulsions and encephalitis). Illness associated with primary HHV-6B infection accounts for 10 to 40% of febrile admissions of young children to pediatric emergency departments. No isolates of HHV-6A independent of HHV-6B have been obtained from immunocompetent children with roseola or other febrile illness.

There may be negative consequences of the failure to diagnose routine HHV-6B infections. One scenario (51, 236, 474) involves a febrile child who is being treated with antibiotics and then breaks out in a rash after a day or two. This might be interpreted as an adverse reaction to the antibiotic and duly noted as such, with that antibiotic and its relatives being stricken from the list of antibiotics available for treating that person for the rest of his or her life. The antibiotic reaction may in fact have been a normal presentation of primary HHV-6B infection. Thus, even though primary infection with HHV-6B has few sequelae, definitive diagnosis of the infection is in the patient's best interest.

In addition to the study of primary infection in children, HHV-6A and HHV-6B have been studied as possible cofactors in other clinical situations. Herpesviruses are common opportunistic pathogens in immunocompromised individuals, and HHV-6A and HHV-6B activity has been detected following renal and liver transplants and bone marrow transplants (BMT). HHV-6 antigens and nucleic acids are widely disseminated in autopsy tissues from AIDS patients. The clinical consequences of these findings are under study. Segments of HHV-6 DNA can transform cells to a malignant phenotype in vitro, and it is possible that the virus plays an etiologic role in Hodgkin's disease (HD) and other malignancies.

An underappreciated aspect of HHV-6 biology is its commensal presence in brain tissue. The implications of this observation are not known. Several recent reports indicate that HHV-6 activity and distribution in the central nervous system (CNS) are altered in multiple sclerosis (MS) patients (87, 540).

NOMENCLATURE AND CLASSIFICATION

The brief history of HHV-6 nomenclature provides a clear reminder that science is done by humans. There have been changes in names and suggested classification, and there have been spirited discussions and arguments about what to call the variant groups. Some issues remain to be settled.

HHV-6A and HHV-6B are members of the *Betaherpesvirinae* subfamily, in the *Roseolovirus* genus along with HHV-7 (Fig. 1). This classification is based on the relatively high level of sequence conservation and general genetic colinearity between these viruses and HCMV (a well-characterized betaherpesvirus), in comparison to the generally lower level of genetic similarity to alpha- or gammaherpesviruses. The genetic relationships of HHV-6A and HHV-6B with the other herpesviruses are discussed in more detail in the section on molecular biology, below.

Shortly after the discovery of HHV-6, several laboratories described the isolation of viruses that appeared to be different strains of the same virus, in that their DNA hybridized specifically with a cloned DNA fragment from one of the primary isolates. Early on, it was noted that in hybridizations of this fragment with *Hin*dIII-digested viral DNA, either of two profiles were detected: a single 8.5-kb band or a 5.4-kb band plus a 23-kb band (50, 244, 268), but the significance of these differences was not evident. In a comparison between two isolates, strains Z29 and U1102 reacted differently with some MAb, and while strain U1102 replicated in the T-cell lines J JHAN and HSB-2, strain Z29 did not (545). Given that only two isolates were compared, the only conclusion possible was that the strains differed in these properties.

Three important papers on the subject of HHV-6 strain grouping were published independently in 1991. Each reported comparisons of several properties across collections of HHV-6 strains. All three papers reported that the collections of HHV-6 isolates segregated into two groups that were consistent from marker to marker. Aubin et al. (35) compared restriction endonuclease profiles from large genomic segments and nucleotide sequences from a 163-bp segment and found two groups, typified by strains SIE and HST. Strains included

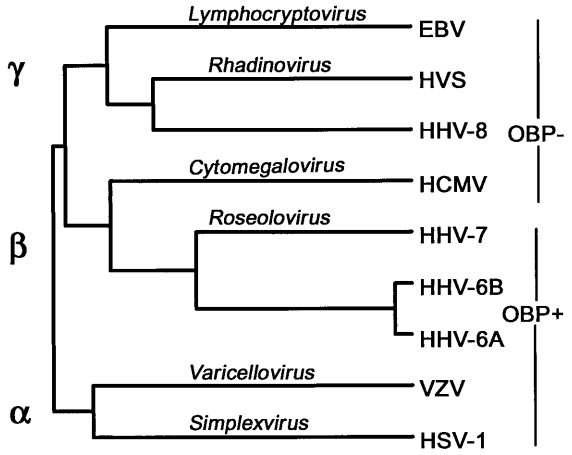

FIG. 1. Genetic relationship of HHV-6A and HHV-6B to other herpesviruses. gH amino acid sequences were aligned by PILEUP (127) with a gap weight of 3 and a length weight of 0.1. The dendrogram represents clustering of the sequences based on their similarity; vertical branch lengths are proportional to the distance between the sequences. α, β, and γ are the herpesvirus subfamilies. Genera within each subfamily are indicated. The close relationship between HHV-6A and HHV-6B is evident, as are the progressively larger distances from these viruses to the other betaherpesviruses, HHV-7, and then HCMV. The viruses indicated as OBP+ encode homologs of the HSV-1 UL9-encoded origin binding protein; their origins of lytic DNA replication are highly conserved. The viruses indicated as OBP− do not encode homologs of this protein, and their origins of lytic DNA replication share little similarity with those of the OBP+ viruses. References for the sequences are as follows: EBV, 39; HVS (herpesvirus saimiri), 16; HHV-8, 368; HCMV, 92; HHV-7, 381; HHV-6B, 317; HHV-6A, 200; VZV, 123; HSV-1, 357.

in group 1 were isolated from immunocompromised adults, and those in group 2 were isolated from children. Ablashi et al. (3) found that isolates that grew in HSB-2 and Sup T1 but not Molt-3 cells reacted with all MAb directed against strain GS and shared identical restriction endonuclease profiles, while the isolates that did not grow in HSB-2 and Sup T1 cells but grew in Molt-3 cells reacted with the same subset of strain GS-derived MAb and shared identical restriction endonuclease profiles that differed from those of the other group. The authors proposed that HHV-6 isolates be divided into group A (GS-like) and group B (Z29-like). Schirmer et al. (455) compared whole-genome restriction endonuclease profiles and reactivity with a panel of MAb among a collection of HHV-6 isolates. The isolates that shared restriction endonuclease profiles with strain Z29 had similar reactivity with the panel of MAb. With the exception of strain Z29, which was isolated from an AIDS patient, the Z29-like viruses were all obtained from ES patients. The authors suggested that reclassification of HHV-6 strains be considered.

There was sufficient overlap in the collections studied in these papers to suggest that the two groups of isolates described in each of the three papers were in fact the same groups. Subsequent studies confirmed this and extended the results to much larger collections (34, 89). As it stands, over 100 HHV-6 isolates have been analyzed by a combination of restriction endonuclease analysis, cell tropism, and reactivity with panels of MAbs (3, 128, 131, 137, 455); except for isolates that appear to be mixtures of the two variants (3, 131), all have been unambiguously assigned to one of the two groups.

While it is clear that HHV-6 isolates segregate into two distinct groups, it is also clear that the two groups are closely related. Members of both groups infect primary CD4-positive T cells. Although a subset of MAb derived against a member of one or the other group do not cross-react between the groups, many MAb do. Much of the human immune response is cross-reactive between the groups, and polyclonal rabbit antibodies to either cells infected with a member of one group or a purified structural protein from a member of one group react strongly with their counterparts from the other group (89, 406). The genomes cross-hybridize efficiently (316, 455). The nucleotide sequence identity ranges from 75 to 97%, depending on which gene is being compared, with identities being in the vicinity of 95% for most genes (34, 35, 101, 197, 200, 315, 317, 323, 406, 554). Notably, both groups encode closely related homologs of the parvovirus *rep* gene at equivalent genomic locations (425, 509); the next most closely related herpesvirus, HHV-7, does not encode such a gene (381).

The clear evidence for two closely related yet distinct groups of HHV-6 isolates has led to a nomenclature conundrum. Do the two groups warrant distinct names? Do the two groups constitute distinct herpesvirus species? The issue was discussed in special sessions at international herpesvirus meetings, and a consensus letter from the community on the subject was published in 1993 (2). The letter described the available evidence and concluded that the groups should be recognized as HHV-6 variants A (strain GS- and U1102-like) and B (strains Z29- and HST-like). It was suggested that it was premature to recognize the variants as distinct species because of their close relationship (closer than any other pair of recognized herpesvirus species) and the gaps in our understanding of the epidemiology of the variants.

In the intervening 4 years, much has been learned, and we would like to describe our reasons for proposing that the issue of recognizing the variant groups as distinct species be reconsidered. We preface the discussion with the definition of herpesvirus species as put forth by the Herpesvirus Study Group of the International Committee on Taxonomy of Viruses (ICTV):

> Related viruses could be classified as distinct species if (a) their genomes differ in a readily assayable and distinctive manner across the entire genome (e.g., restriction endonuclease cleavage site patterns obtained with many enzymes) and not merely at a specific site (e.g., small number of genes or small number of restriction endonuclease sites) and (b) if the virus can be shown to have distinct epidemiologic and biologic characteristics (436).

Has this definition been met? With respect to item "a" from the ICTV definition, which relates to genomic differences, we think the answer is, "Yes." Evidence for this includes the identification of variant-specific nucleotide sequence differences in fragments from diverse segments of the respective genomes and the manifestation of these differences in the markedly distinct restriction endonuclease fragment profiles of each variant. These are perhaps most easily appreciated in the whole-genome restriction endonuclease profiles presented in the studies of Schirmer et al. (455). The sequence variation is logically the source of the variation in reactivity with panels of MAb directed against a variety of viral proteins.

An important question to consider, given the high degree of similarity between the HHV-6 variants, is whether there is any evidence for natural recombination between them, which would suggest overlapping or shared biologic niches and the absence of speciation. Points of comparison would be the EBV variants and interstrain variation among HCMV isolates. In the case of EBV, viruses have been identified that are EBV-1 at some loci and EBV-2 at others (314), suggesting that there is a biological gradient between the variants that precludes their recognition as distinct species. For HCMV, the evidence when considered in its parts (single genes) appears to indicate

the presence of defined strain groups, but when the totality of evidence (multiple genes) is considered, strains that sort into a given group when the sequence of one gene is analyzed, sort into other groups when other genes are analyzed, there being no constancy of the sorting from marker to marker (97, 98, 304). There is no reported evidence of a genetic gradient between HHV-6A and HHV-6B; all isolates that have been characterized for more than one marker have been unambiguously assignable to one or the other variant.

Do the variants have "distinct epidemiologic and biologic characteristics" (item "b")? As mentioned above, A variants replicate preferentially in HSB-2 and J JHAN cells and B variants replicate preferentially in Molt-3 cells. There is a nearly absolute association of B variants with ES (128, 131, 137, 405, 455); the two A variants found were each present in the ill child simultaneously with the B variant (131). Of HHV-6 strains detected in BMT recipients, 70 (87.5%) of 80 were B variants, 6 (7.5%) were A variants, and 4 (5%) were mixtures of HHV-6A and HHV-6B (147, 178, 254, 458, 528, 539). As recently elegantly summarized by Di Luca et al. (135), there are significant variant-specific differences in the frequencies of detecting HHV-6 DNA in (i) peripheral blood mononuclear cells (PBMC) of healthy donors, CFS patients, and HIV-infected patients; (ii) lymph nodes from patients with various conditions; and (iii) various tissues. In most of the examples, the B variant was detected most frequently, with HHV-6A being detected more frequently than HHV-6B in healthy skin, KS lesions, KS-derived cell lines, and primary fibroblast cultures, suggesting a preferential tropism of HHV-6A for skin. The distinctions between the variants were less marked in lung tissue specimens, where 22 (65%) of 34 specimens were PCR positive for both variants, 2 (6%) were positive for HHV-6A, and 10 (29%) were positive for HHV-6B (115). Although there is some overlap in tissue distribution and disease associations, the variant-specific differences in cell tropism, disease associations, and tissue distribution seem sufficient when considered together to constitute "distinct epidemiologic and biologic characteristics."

We are of the opinion that sufficient new information relating to the biology and epidemiology of the HHV-6 variants has accumulated to allow reconsideration of the classification of the HHV-6 variants as distinct species. As we were reminded by Di Luca et al. (135), the herpesvirus classification system is intended to allow researchers and clinicians to anticipate the properties and pathogenic potentials of new isolates (436). Thus, whether or not the species threshold is deemed to have been met, the differences between the variants are real and require recognition for us to fully appreciate the subtleties of the specific niches that the viruses occupy. In this review, we have highlighted areas where variant-specific differences have been noted. In addition, to the extent that we could manage, we have treated the variants as separate entities, even in areas for which variant-specific differences have not been (and might not be) identified. Although this has led to some instances of more difficult phraseology, we do not consider the absence of evidence for differences to mean the absence of differences, and we hope to stimulate continued study of the similarities and differences between the variants. We also hope that a by-product of this and other work describing variant-specific differences will be motivation of authors of HHV-6-related papers in the future to be clear in specifying which variant was studied, isolated, or otherwise detected.

CELLULAR BIOLOGY

Ultrastructure and Morphogenesis

Virions of HHV-6A and HHV-6B, as for all herpesviruses, have four main structural elements: an electron-dense core, a capsid with icosahedral symmetry, a tegument (the less highly structured material occupying the space between the capsid and the envelope), and an outer envelope in which virally encoded glycoproteins and integral membrane proteins are embedded (reviewed in reference 437). The assembly process leading to mature HHV-6 particles begins in the nucleus, where nucleocapsids are assembled. HHV-6 capsids have a diameter of 90 to 110 nm (53, 562). After capsid assembly, the tegument is acquired while still in the nucleus by a series of envelopment and deenvelopment steps which have not been fully characterized (385, 434). The tegument may be acquired in a novel structure, the tegusome, which is a membrane-bounded spherical compartment, apparently of cytoplasmic origin, that is present in the nuclei of HHV-6-infected cells (434). Fully tegumented capsids, with a diameter of approximately 165 nm, appear to be released into the cytoplasm via fusion of the tegusome with the nuclear membrane (434, 562). HHV-6 morphogenesis has the unique feature of cytoplasmic accumulation of fully tegumented capsids with a prominent and well-demarcated tegument; these tegumented cytoplasmic capsids appear to acquire their envelope by budding into cytoplasmic vesicles. Mature virions are approximately 200 nm in diameter (53, 562) and are released by exocytosis (434).

Cell Tropism

In vitro. HHV-6A and HHV-6B replicate most efficiently in vitro in activated primary T cells, and several isolates have been adapted to grow efficiently in continuous T-cell lines. Of the two most widely used strains of HHV-6A, strain GS is most commonly propagated in the T-cell line HSB-2 and strain U1102 is most commonly propagated in J JHAN cells. HHV-6B(Z29) is grown most often in primary lymphocytes and has been adapted for growth in the Molt-3 T-cell line. While T cells are most widely used for propagation of HHV-6A and HHV-6B, cell lines of neural, epithelial, and fibroblastic origin have different levels of permissivity for HHV-6 growth in vitro (6, 86, 94, 213, 325), although none of these cells are in common use for routine propagation of the virus. With few exceptions, virus isolation procedures have relied on cultivation of patient PBMC or cocultivation with primary adult PBMC or umbilical cord blood lymphocytes. HHV-6 can infect primary natural killer (NK) cells harvested from PBMC (341).

In vivo. The in vivo host tissue range of HHV-6 is broader than its in vitro host range might suggest and includes lymph nodes (309), lymphocytes (112, 119, 243), macrophages and monocytes (288), kidney tubule endothelial cells (388), salivary glands (174, 296, 424), and CNS tissues, where viral gene products have been localized to neurons and oligodendrocytes (87, 329, 330).

Takahashi et al. (497) sorted lymphocytes obtained from children with primary infections during episodes of ES and found that virus was recovered most abundantly from $CD4^+$ cell populations. Because these isolates were obtained during ES, they were probably HHV-6B. Four weeks following primary infection, virus could be recovered only from macrophages (288). As discussed below, this finding suggests that macrophages are a potential repository for latent infection.

Lytic Replication Cycle

There are many gaps in our knowledge about the replication cycles of HHV-6A and HHV-6B. A specific receptor on target cells has not been identified for either virus. Although both viruses have a primary tropism for $CD4^+$ T cells, CD4 does not appear to be the receptor. Thus, HHV-6A replication is not inhibited by soluble CD4 or by antibodies to CD4, under conditions in which HIV-1 replication is inhibited (338). Expression of CD4 on cells that do not normally express it did not render the cells susceptible to infection by HHV-6B (162).

The route of entry into the cell and the mechanism of migration of the capsid to the nucleus are not known, nor are the mechanisms of unpackaging the DNA and transporting it to the cell nucleus. Within 24 h after infection with HHV-6B, the rate of cellular protein synthesis is higher in infected cells than in mock-infected cells (43, 56, 134), and by 65 h after infection, host cell DNA synthesis is shut off and viral DNA synthesis is under way (134). Newly replicated virus is first detected at 72 h after infection (58). Virion maturation and egress are described in the section on ultrastructure and morphogenesis, above.

Effects on Cells

Herpesviruses can have profound effects on host cell metabolism. For example, HSV-1 rapidly shuts off host cell macromolecular synthesis, induces nucleoli to disaggregate, and causes chromatin to marginate (reviewed in reference 437). In contrast, HCMV stimulates both protein synthesis and cell division (219, 240, 478, 573). Different aspects of the virus-cell interaction have been studied for HHV-6A than for HHV-6B; it remains to be seen whether the findings for one virus apply to the other.

As indicated by background levels in radiolabeled-protein profiles, HHV-6A does not shut off host cell protein synthesis (42). Infection of mononuclear cells led to increased expression of alpha interferon (IFN-α) (270), CD4 (335, 340, 341), interleukin-1β (IL-1β), and tumor necrosis factor alpha (TNF-α) but not IL-6 (165). The induction of CD4 rendered previously refractory lymphocytes susceptible to infection with HIV-1 (335); this effect may have pathogenic implications.

HHV-6B shuts off host cell DNA synthesis within 65 h of the initiation of infection (134). In parallel with the shutoff of host DNA synthesis, there is a broad stimulation of host cell protein synthesis at times when protein synthesis has nearly ceased in uninfected primary lymphocytes (56). At 3 to 5 days after infection, infected-cell protein profiles are similar to uninfected-cell protein profiles immediately following phytohemagglutinin stimulation, with the major difference being the higher level of synthesis in the infected cells. The net effect is a continuation of protein synthesis at levels even greater than those seen in freshly stimulated lymphocytes, accompanied by an inhibition of cell division. Thus, it appears that the virus has disrupted a component of the cell cycle that links cytoplasmic growth with cell division. The decoupling of cell growth from cell division may lead to the commonly observed effect in HHV-6-infected cell cultures of the production of large, refractile cells.

MOLECULAR BIOLOGY

Genome

Structure. The genomic architecture shared by HHV-6A, HHV-6B, and HHV-7 (142, 318, 351, 443) is unique among the HHVs and resembles that of channel catfish virus (103). HHV-6 genomes are composed of a linear, double-stranded DNA molecule bounded by directly oriented terminal repeats (DRs) (Fig. 2). The unit-length molecule ranges from approximately 162 to 170 kb and is composed of a 143-kb unique long (U) segment bracketed by direct repeats, DR_L (left) and DR_R (right), that can vary in length from 8 to 13 kb upon passage in vitro (200, 318, 351, 378, 510). The complete genome sequence of HHV-6A(U1102) has been determined; its U segment is 143,147 bp and is flanked at each terminus by an 8,087-bp DR (200) for an overall length of 159,321 bp. The DRs in the sequenced virus are probably shorter than in the wild-type virus from which they were derived (405). It will be interesting to compare this sequence with sequences of DRs derived from wild-type viruses that have not undergone passage in culture. The G+C content is not constant across the genome, averaging 41% over the unique segment and 58% in the DRs (199, 200, 318, 508), for a net value of 43%. Uneven base composition has been observed in other sequenced herpesviruses; for example, the G+C content of the unique region of the herpesvirus saimiri genome is 36%, compared with 71% in the terminal repeats (16).

An interesting feature of the HHV-6 genome is that it contains reiterations of the hexanucleotide $(GGGTTA)_n$ near the ends of the DRs (199, 274, 316, 508) (Fig. 2). Although this hexanucleotide is present in both variants, the context of the reiterated sequences within DR in HHV-6A and HHV-6B differs; HHV-6A contains additional repeat sequences not found in HHV-6B (508). This sequence may have been acquired from the host cell since it is present at the telomeres of vertebrate chromosomes (362). This sequence is also found near the termini of HHV-7, a closely related virus (459), and at the junction region between the internal inverted repeats IR_S-IR_L of Marek's disease virus (a lymphotropic alphaherpesvirus of chickens) and herpesvirus of turkeys (273). The function of $(GGGTTA)_n$ is not known, but it has been hypothesized to play a role in DNA replication and in maintenance of the viral chromosome as a self-replicating episome in latently infected cells (199, 233, 508). Since $(GGGTTA)_n$ is located near but not at the genomic termini, it seems unlikely that the repeat array is involved in maintaining or protecting the integrity of the ends of the viral genome during DNA replication (508).

The conserved terminal *cis*-acting herpesvirus packaging signals, *pac*-1 and *pac*-2, are present in HHV-6A and HHV-6B, flanked by imperfect and perfect iterations of GGGTTA (199, 508) (Fig. 2). During DNA replication, a head-to-tail arrangement probably occurs, juxtaposing the *pac*-1 and *pac*-2 signals in the correct orientation, creating a favored cleavage site for unit-length molecules. Single copies of the telomere-like sequence are distributed throughout the genome and exhibit marked polarity, TAACCC to the left of the origin for lytic-phase replication (*ori*Lyt) and the complement, GGGTTA, to the right of *ori*Lyt (199). Three other sets of reiterations, R1, R2, and R3, are located at or near the major immediate-early loci (Fig. 2); R3 may play a role as a transcriptional enhancer (200, 350, 351).

As with other herpesviruses, the HHV-6A and HHV-6B genomes are densely packed genetically, with very little noncoding DNA (Fig. 2) (200, 315, 317). HHV-6A and HHV-6B share amino acid similarities as well as an overall similar gene organization with other betaherpesviruses, such as HCMV (153, 238, 301, 380) and HHV-7 (142, 381). Herpesviruses encode a set of conserved genes that are grouped into seven gene blocks, with each block being composed of two to eight genes (92, 200) (Fig. 3). The gene blocks have different genomic location, order, and orientation in the different herpesvirus subfamilies, although genes within a gene block maintain order and transcriptional polarity. These conserved gene

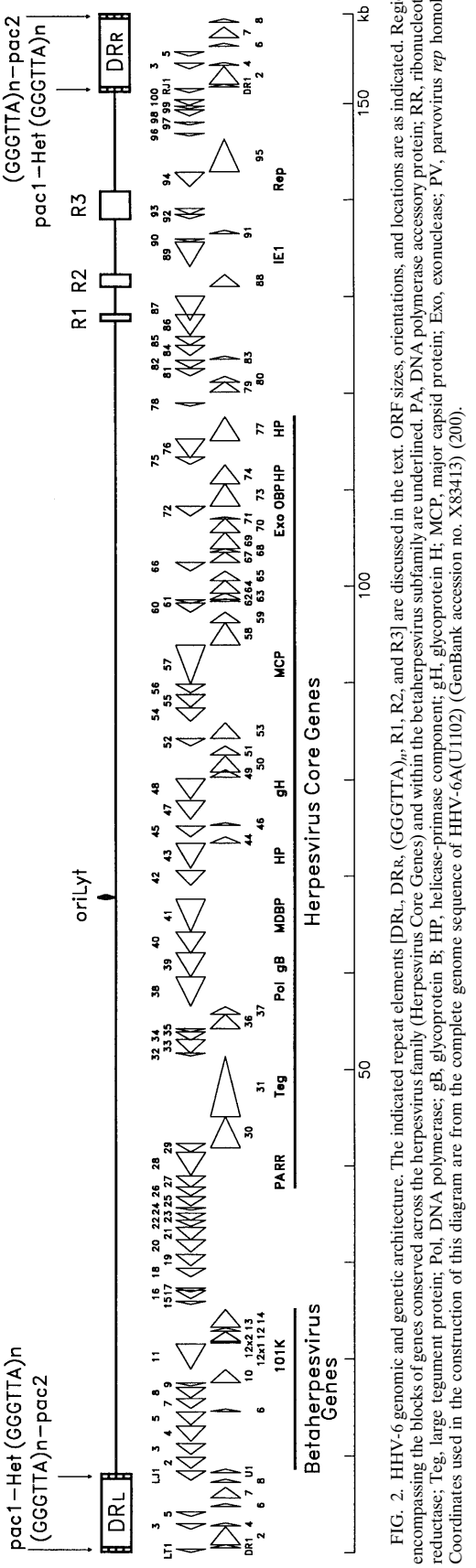

FIG. 2. HHV-6 genomic and genetic architecture. The indicated repeat elements [DR_L, DR_R, $(GGGTTA)_n$, R1, R2, and R3] are discussed in the text. ORF sizes, orientations, and locations are as indicated. Regions encompassing the blocks of genes conserved across the herpesvirus family (Herpesvirus Core Genes) and within the betaherpesvirus subfamily are underlined. PA, DNA polymerase accessory protein; RR, ribonucleotide reductase; Teg, large tegument protein; Pol, DNA polymerase; gB, glycoprotein B; HP, helicase-primase component; gH, glycoprotein H; MCP, major capsid protein; Exo, exonuclease; PV, parvovirus *rep* homolog. Coordinates used in the construction of this diagram are from the complete genome sequence of HHV-6A(U1102) (GenBank accession no. X83413) (200).

products include structural components of the virion, such as MCP, gH, and the large tegument protein. Others are enzymes required for DNA replication and nucleotide metabolism, such as the major DNA binding protein (MDBP), DNA polymerase, and uracil-DNA glycosylase. As illustrated in Fig. 2 and 3, HHV-6 also encodes an additional gene block, the β-genes, which have been found only in HCMV and are absent from the genomes of the human alpha- and gammaherpesviruses (200, 382). Many of these genes belong to the US22 family of proteins, which are present in scattered clusters throughout the HCMV genome (92). Genes belonging to this family contain one or more of three conserved amino acid motifs (92), and open reading frames (ORFs) related to this gene family are also present in HHV-7 (142, 381). The biological function of the products of the US22 gene family remains unclear, although two members from HCMV, TRS1 and IRS1, can transactivate gene expression (475). The HHV-6A(U1102) genome encodes at least 102 ORFs which are likely to encode a gene product (200) (Fig. 2). Of the 102 genes, 70 have an HCMV homolog. These genes, spanning U2 through U86, are located in the unique region of the HHV-6A genome and are arranged in the same order and orientation as genes in the HCMV UL region. HHV-6 genes for which a function has been assigned either experimentally or by analogy to other herpesviruses are listed in Table 2.

DNA replication. The elements required for lytic HSV-1 DNA replication are an origin for lytic-phase replication and seven viral gene products: the MDBP, the DNA polymerase, a polymerase-associated protein, and an origin binding protein (OBP), as well as a helicase-primase complex composed of three proteins encoded by the HSV-1 UL5, UL8, and UL52 genes (reviewed in reference 437). HHV-6 encodes homologs to all seven gene products. An interesting feature is that HHV-6 encodes a sequence homolog of the HSV-1 OBP, which had been found only in alphaherpesviruses (200, 234, 302, 315, 380). The HHV-6 OBP, like other alphaherpesvirus-encoded OBP homologs, has greater amino acid sequence similarity to other herpesvirus OBPs in the N-terminal helicase region than in the C-terminal DNA binding region (302, 317). HHV-6 has an origin for lytic-phase DNA replication that, as in HSV-1, VZV, and HCMV, is located upstream of the gene encoding the MDBP homolog (21, 480, 535) (Fig. 2). The HHV-6B(Z29) origin lies in a unique 1,400-bp region that has no obvious protein-coding capacity and is located between the genes encoding the MDBP and the HCMV UL69/HSV-1 ICP27 homolog (129, 317, 473). These *ori*Lyt sequences have been functionally defined by using transient-transfection assays with plasmids containing different portions of the HHV-6B(Z29) *ori*Lyt (129); a region of approximately 400 bp was identified as the minimal active region.

The HHV-6B(Z29) *ori*Lyt is located in a genomic region that includes two binding sites, OBP-1 and OBP-2, for the HHV-6B(Z29) OBP. The OBP sites are arranged with dyad symmetry, flank an AT-rich spacer, and are within the minimum essential origin region. This region is adjacent to a larger AT-rich imperfect direct repeat of about 195 bp that has been proposed to act as a DNA-unwinding element (129). Both OBP sites are required for DNA replication in transient-transfection assays (130). Functional assays have shown that the HHV-6B(Z29) OBP does not bind to the HSV-1 binding site and the HSV-1 OBP does not bind to the HHV-6B(Z29) sequence (234). Competitive electrophoretic mobility shift assays (EMSA) with mutated HHV-6B(Z29) OBP-1 determined that the consensus binding site is YGWYCWCCY (where Y is T or C and W is T or A), which is different from the HSV-1 consensus binding site, YGYTCGCAC (235). Studies mapping

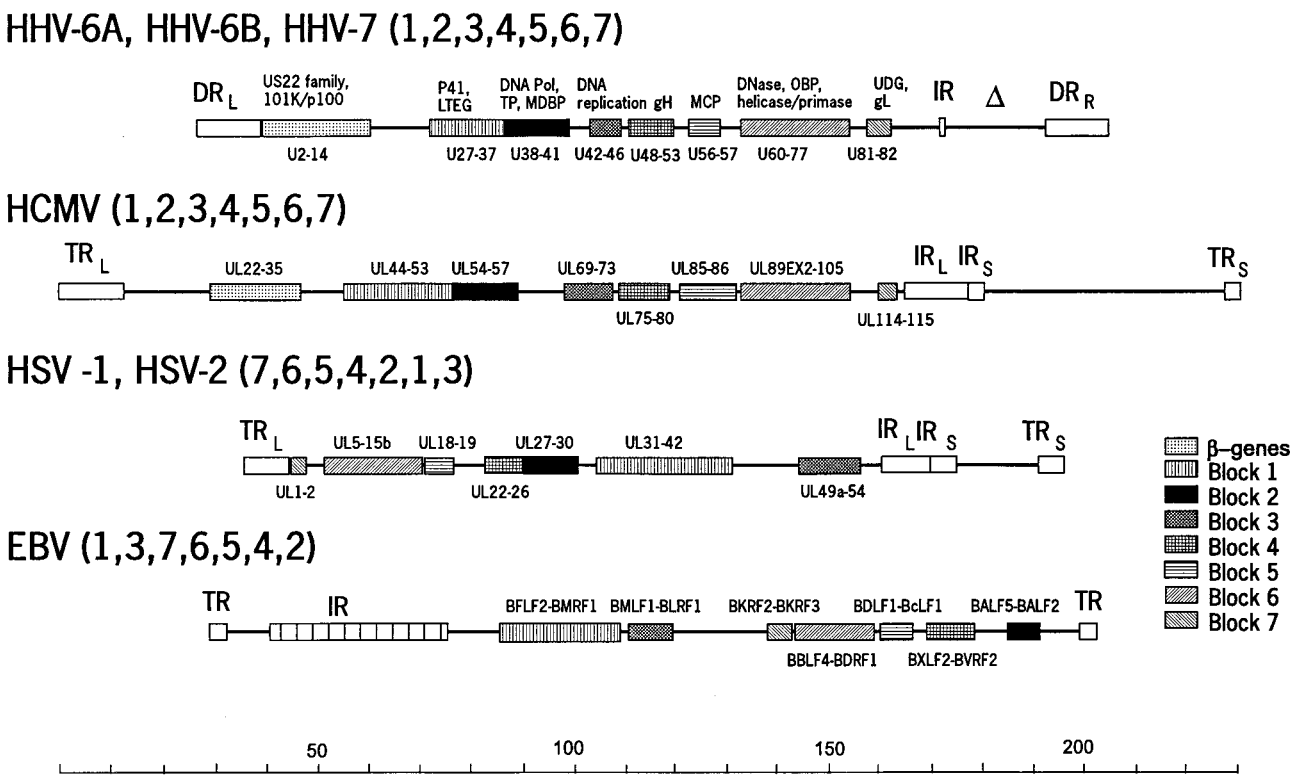

FIG. 3. Conserved herpesvirus gene blocks (adapted from references 92, 200, 233, and 364). Prototype arrangements of the HHV-6A/6B/7, HCMV, HSV-1, and EBV genomes are shown. Beneath the schematic diagrams is a scale in kilobase pairs. The seven conserved herpesvirus sequence blocks (Block 1 through Block 7) plus an additional sequence block (β-genes) that is found only in the betaherpesviruses are diagrammed. The order of the gene blocks on each prototype genome is shown after the virus name. The ORFs defining the boundaries of each conserved sequence block are indicated. Gene nomenclature is from the following references: HHV-6A, 200; HCMV, 92; HSV-1, 357; and EBV, 39. The structures of genomic termini are also shown. DR_L (left) and DR_R (right) denote direct repeats. IR denotes an internal repeat. TR_L, IR_L, IR_S, and TR_S denote inverted repeats flanking the long or L component and the short or S component, respectively. TR are direct terminal repeats. Some of the gene products found in these sequence blocks are indicated above the HHV-6/7 genome. The 7-kb segment that is present in HHV-6A and HHV-6B and absent in HHV-7 is indicated by Δ. Abbreviations: 101K/p100, virion structural protein; P41, polymerase-associated factor; LTEG, large tegument protein; DNA Pol, DNA polymerase; TP, transport/capsid assembly protein; gH, glycoprotein H; MCP, major capsid protein; UDG, uracil-DNA glycosylase; gL, glycoprotein L.

the minimal DNA binding domain of the HHV-6B(Z29) OBP by either EMSA or Southwestern blotting gave different results (235). The DNA binding domain of OBP was mapped to amino acids 482 to 770 by EMSA and to amino acids 657 to 770 by Southwestern blotting. The smaller region as determined by Southwestern blotting may encompass the sequence that contacts DNA directly, whereas the sequence upstream may confer stability or accessibility to the binding domain. The proposed DNA binding domain is the most conserved segment in the otherwise not highly conserved carboxy-terminal half of OBP.

Interstrain variation. The genomes of variants A and B are genetically colinear, cross-hybridize extensively, and have identical sizes and base compositions (318, 351). Nonetheless, there are sufficient sequence differences that HHV-6A and HHV-6B have characteristic restriction endonuclease profiles which allow for unambiguous segregation into variant specific patterns (3, 34, 35, 455). Nucleotide sequence variation between HHV-6A and HHV-6B is 1 to 5% in the center of the genome, where conserved core genes are present (2, 34, 196). The interstrain variation in coding sequences between isolates of HHV-6A and HHV-6B is less pronounced than that observed among HCMV isolates (99, 181, 196). For instance, the DNA polymerase, glycoprotein B (gB), phosphotransferase, and MDBP genes from a sample of HHV-6B that was present in the brain of an MS patient shared 99.9% amino acid identity with the corresponding HHV-6B(Z29) sequence (87). The sequence variation among different strains of HCMV in the genes coding for gB and gH is small (approximately 5%), although each coding sequence contains variable loci which allow for the segregation of gB and gH into four and two different genotypes, respectively (99, 181). The nucleotide sequence identity between HCMV strains within each group is 98 to 99%, whereas the similarity between strains in different groups is 88 to 95%. An important distinction is that the HCMV strain groupings are different depending on which gene is analyzed. Heterogeneity within an HHV-6 variant group can differ depending on which gene is being analyzed. For example, when a 210-bp region of the U47 gene was analyzed, variant A viruses differed by 2% whereas variant B viruses were identical in this region (196).

Several genomic regions in HHV-6 stand out because of nucleotide sequence differences that exceed 10%. These include the immediate-early region, which differs by as much as 25% (101, 554), and the gene encoding the highly immunoreactive virion protein, which differs by 10% (406). Extensive nucleotide variation is also present between the two main types of EBV that circulate in the human population, EBV-1 and EBV-2. The major differences between the types are located in the genes expressed during latency, such as those encoding the nuclear proteins (EBNAs) and the small RNAs (266). For example, the amino acid sequence of the EBV-1 EBNA 2, EBNA 3A, and EBNA 3B, and EBNA 3C differ from the corresponding EBV-2 products by 47, 16, 20, and 28%, respec-

TABLE 2. Selected HHV-6 genes and similarity implied or observed functions

HHV-6 ORF	Function
DR7	Transforms NIH 3T3 cells, HIV LTR transactivator
U11	101K, p100, antigenic virion protein
U12	G-protein coupled receptor
U16	Immediate-early gene (IE), HIV LTR transactivator
U27	Polymerase processivity factor (PA), HIV LTR transactivator
U28	Large ribonucleotide reductase subunit
U29	Capsid assembly and DNA maturation
U31	Large tegument protein
U38	DNA polymerase
U39	Glycoprotein B (gB)
U40	Transport/capsid assembly (TP)
U41	MDBP
U43	Helicase/primase complex (HP)
U48	Glycoprotein H (gH)
U51	G-protein coupled receptor
U57	Major capsid protein (MCP)
U70	Alkaline exonuclease
U72	Glycoprotein M (gM)
U73	OBP
U74	Helicase/primase complex (HP)
U77	Helicase/primase complex (HP)
U81	Uracil-DNA glycosylase
U82	Glycoprotein L (gL)
U83	Intercrine cytokine
U89	Immediate-early gene (IE), HIV LTR transactivator
U94	Parvovirus *rep* homolog, HIV LTR regulator

tively (451). A limited amount of sequence information has been reported for additional EBV-1 and EBV-2 genes. For example, identity in the noncoding DNA adjacent to the EBNA-3 ORFs is 96%, and it is over 95% in the BZLF1 ORF (247, 451). Aside from these genes, the genomes have very similar restriction endonuclease profiles, exhibiting restriction endonuclease polymorphism characteristic of strain differences with no segregation into type-specific patterns.

Intrastrain heterogeneity. Heterogeneity also occurs within HHV-6 isolates. Regions of increased divergence, including both insertions and deletions, map to regions with repetitive elements. In HHV-6B(Z29), intrastrain heterogeneity maps to a region near the left end of both copies of DR and is manifested by the loss of 3 to 4 kb from each DR upon serial passaging in cell culture (318, 405). The variable portion of the genome is known as the *het* region; variation among other isolates has also been mapped to this region (178, 199, 351, 508). The events that lead to such heterogeneity have not been identified, but the process may be related to viral replication.

Relationship of HHV-6 with other herpesviruses. HHV-6 was initially grouped as a lymphotropic virus or gammaherpesvirus, as exemplified by EBV, based on its ability to infect lymphocytes (324, 450). However, early studies of HHV-6 DNA indicated that it was more closely related to HCMV, a betaherpesvirus (152, 301), and the virus is now classified as a member of the *Roseolovirus* genus of the betaherpesvirus subfamily (Fig. 1). Since the sequence for the HHV-6A(U1102) genome has been determined, a complete analysis of the coding content is available (200) (Fig. 2). Of HHV-6A(U1102) genes, 67% have HCMV counterparts, on the basis of either having amino acid similarity or having positional similarity. HHV-6 is even more closely related to HHV-7 than to HCMV, based on limited serologic cross-reactivity, DNA hybridization, and nucleotide sequence similarity (52, 55, 142, 171, 179, 381) (Fig. 1). The MCP of HHV-6A(U1102) shares 61.3% amino acid identity with the MCP of HHV-7 (373) and 43.8% amino acid identity with the HCMV counterpart (321, 505) but has less than 30% amino acid identity with the MCP of EBV, VZV, and HSV (321). This relatedness is similar to that of other herpesviruses in a given subfamily. For example, HSV-1 and VZV share 52% amino acid identity in the MCP (357).

Some HHV-6 genes have no known counterpart in other herpesviruses. In HHV-6A(U1102), 25 unique genes are located either in the DR or near the ends of the unique segment; in some of these regions, splicing occurs (200, 408). Two of the more intensively studied HHV-6 unique genes are the adeno-associated virus type 2 (AAV-2) *rep* gene ($rep_{AAV\text{-}2}$) homologs (rep_{H6}) and the gene encoding the spliced glycoprotein gp82/105 (407, 408, 425, 509).

The $rep_{AAV\text{-}2}$ homolog is encoded by HHV-6A(U1102) ORF U94 (Fig. 2) and is present in a similar genomic location in HHV-6A and HHV-6B (425). Its product Rep_{H6} is more closely related to the $rep_{AAV\text{-}2}$ protein ($Rep_{AAV\text{-}2}$) than to the homologous proteins encoded by other parvoviruses (511). It is worth emphasizing that HHV-7 lacks a $rep_{AAV\text{-}2}$ gene homolog, and thus this gene may be a marker of evolutionary divergence between HHV-6 and HHV-7 (381). The Rep protein is essential for AAV-2 DNA replication and possesses site-specific ATP-dependent endonuclease and helicase activities (232). The functions of the *rep* gene and its product in the life cycle of HHV-6 are unknown. In vitro, rep_{H6} can provide helper function for AAV-2 DNA replication (511). Additionally, rep_{H6} from HHV-6A(U1102) can complement the replication of a *rep*-deficient AAV-2 genome.

Another unique HHV-6 protein is a major envelope glycoprotein which consists of a number of related polyproteins, collectively referred to as the gp82/gp105 complex (407). The role of gp82/gp105 is not known but is likely to be important in the biology of HHV-6 since neutralizing epitopes are present. MAb raised against HHV-6A(GS) are able to neutralize HHV-6A infectivity but are unable to neutralize HHV-6B infectivity (407). The presence of multiple RNA species that hybridize to the gene encoding the neutralizing epitope suggests that the other polypeptides in the gp82/gp105 complex are probably the result of differential splicing among the 12 exons in the gp82/105 gene (408). This is the first reported HHV virion envelope glycoprotein to be composed of multiple species derived from the same gene as a result of differential splicing.

Transcription

During lytic infection, herpesvirus genes are expressed as three kinetic classes (reviewed in reference 437). The α, or immediate-early (IE), genes are transcribed with no requirement for de novo protein synthesis; mRNAs are frequently detectable within 3 h after infection. α proteins regulate the expression of other genes. The second class of genes, the β or early (E) genes, are involved in DNA metabolism and DNA replication. The expression of β genes is dependent on α-gene expression. The third class of genes is the γ, or late (L), genes; the products of these genes are usually involved in virion assembly and as components of the mature virus particle.

Temporal regulation of HHV-6 gene expression is not well understood. Several transcription units have been characterized (406, 408, 425, 454). The most intensively studied region is near the right end of the unique segment of the HHV-6A(U1102) genome and shares characteristics with regions in the betaherpesvirus genomes which code for IE gene products (350, 454). These characteristics include CpG dinucleotide frequency suppression, encoding transcriptional *trans*-activators, and the presence of a complex array of repetitive sequences which contain binding sites for cellular transcription initiation

factors. An IE locus spanning approximately 5 kb of sequence is located at genomic coordinates 132272 through 136826 in HHV-6A(U1102). This locus gives rise to two spliced coterminal RNAs of 3.5 and 4.7 kb plus two other smaller transcripts from the opposite strand. The 3.5- and 4.7-kb species trace back to ORFs U89 and U90, respectively. Only the 3.5-kb species is detected under α conditions; the 4.7-kb transcript is present at late times after infection and initiates from a different promoter located upstream from the repetitive element R3 (Fig. 2) (454). The short transcripts arise from the same promoter and are not expressed during α conditions. The similarity to the HCMV IE region is limited to the most general properties, since there is no similarity at the level of genetic content or pattern of gene expression between this HHV-6A IE locus and the HCMV IE counterpart. Another HHV-6 IE candidate is the B701 gene fragment of HHV-6A(GS) ORF U16 in HHV-6A(U1102), which is located near the left end of the genome (191, 382). This gene is a member of the US22 gene family and shares weak amino acid similarity with UL36 from HCMV, which is an IE gene that regulates gene expression (108). In vitro, B701 can transactivate the HIV-1 promoter and thus may function as a transactivator of HHV-6A gene expression (191).

Transcriptional activity from the gene encoding the virion protein 101K in HHV-6B(Z29) gives rise to a set of transcripts approximately 2.6 kb long. When the termini of these mRNAs species were precisely mapped, two 5′ and two 3′ ends were detected (406). The transcripts originated downstream from either of two TATA boxes located 139 bp apart in the region 5′ to the 101K ORF, with the 5′ species closest to the initiating ATG being more abundant. Of the two 3′ termini, the one with the canonical polyadenylation signal (AATAAA) was used more frequently. The significance of this finding is not known but suggests that these mRNAs may be differentially regulated.

Splicing may also be an important feature of HHV-6 transcriptional regulation. As described for the IE locus, the gene encoding gp82/105 (408) and the rep_{H6} gene are expressed as spliced transcripts (425). The gene encoding HHV-6A(GS) gp82/105 is composed of 12 exons totaling 2.5 kb that spans 20 kb of genomic sequence. Differential splicing is thought to play a key role in the generation of the various mRNA species of the HHV-6A and HHV-6B gp82/gp105 complex (408).

Tight regulation of transcript levels may also be important. The rep_{H6} gene transcript from HHV-6B(Z29) is expressed at very low levels, with maximum expression of 10 copies per cell three days after infection of Molt-3 cells (425). The reason for this tight control is not known, but it appears that overexpression of this gene may be deleterious to the biology of HHV-6.

Characterization of HHV-6 promoters is limited. The HHV-6A DNA polymerase gene promoter is TATA-less, and the major upstream regulatory element required for transcriptional activity is an ATF/CREB transcription binding site (8). A novel 21-bp element located 19 bp upstream of the TATA box is critical for the expression of the HHV-6A U27 gene, the DNA polymerase-associated factor (506). The novel *cis* element contains a C/EBP transcription factor binding site, an overlapping 6-bp direct repeat, and an overlapping 5-bp inverted repeat. The extent to which binding of transcription factors to this element is involved in regulating the expression of the U27 promoter has not been determined.

Infected Cell Proteins

As described above, 102 ORFs with coding potential have been identified in the HHV-6A(U1102) genome sequence (200). Detection of HHV-6-specific proteins is complicated by the fact that HHV-6 does not shut off host cell protein synthesis (42, 43, 56). Instead, host cell protein synthesis increases following infection. This effect appears to be dependent on viral DNA replication because cells infected with UV-irradiated virus showed a proportional decrease in cell number, host protein synthesis, viral DNA, and viral proteins with increased times of UV exposure and there was no increased host cell protein synthesis in cells which were infected in the presence of an inhibitor of viral DNA replication, such as phosphonoacetic acid (56).

To characterize polypeptides present in HHV-6 virus particles, labeled virions were analyzed by electrophoresis. Twenty-nine polypeptides ranging in size from 30 to 280 kDa with a prominent band at 180 kDa were detected (271, 464, 553). A total of 23 to 33 virion polypeptides have been identified for other HHVs (437). When purified virions were treated with a nonionic detergent and separated into envelope and nucleocapsid fractions, the whole virion and nucleocapsid fractions contained a major band of approximately 143 kDa, which corresponds to the MCP of HHV-6 (271, 464, 553). The envelope fraction revealed six polypeptides. HHV-6 envelope proteins include gB, gH, gL, gM, and glycoprotein gp82/105, based on sequence homologs to other herpesvirus envelope proteins and the identification of neutralizing antibodies (200, 322, 407, 499). In other studies, HHV-6 proteins have been identified by using high-titer human sera to immunoprecipitate proteins from cells infected with HHV-6A(GS) (42, 43). When this method was used, 20 polypeptides ranging in sizes from 180 to 26 kDa, with a prominent band at 135 kDa, were identified. This polypeptide was recognized by all sera tested. These studies were done before it was recognized that HHV-6 isolates segregate into two groups; variant-specific differences in protein profiles of whole virions, nucleocapsid, and envelope fractions have not been studied.

Most HCMV-seropositive sera strongly react with an HCMV protein species with an apparent molecular mass of 150 kDa (reviewed in reference 470). This protein, pp150, is a major component of the tegument, is highly phosphorylated, and is the product of the UL32 ORF. Most human sera with HHV-6-specific antibodies react strongly with a protein with an apparent molecular mass of 101 kDa (101K) (553). This protein is a marker for HHV-6 infection, since sera lacking HHV-6-specific antibodies but seropositive for another herpesvirus fail to react with this protein. The HHV-6A counterpart, p100, has an apparent molecular mass of 100 kDa and, like 101K, is a major constituent of HHV-6 virions (379). Both p100 and 101K have limited similarity to the amino-terminal one-third of the HCMV pp150 and share an 81% amino acid sequence identity (406). Thus, this protein is an excellent candidate for the development of a variant-specific serodiagnostic assay.

In HHV-6A-infected cells, like in all other herpesvirus-infected cells, gH can form complexes with gL, and the gH-gL complex is probably involved in viral spread from cell to cell (322). The domain required for protein-protein interaction between gH and gL is located at the N terminus of gH, a region that is well conserved among betaherpesviruses (22). Interestingly, there appears to be functional correspondence as well, given that the HCMV homologs to gH or gL can substitute for HHV-6 glycoproteins in complex formation (22).

Transcriptional Activation by HHV-6

HSV-1, EBV, and HCMV encode gene products which are able to *trans*-activate in vitro the HIV-1 long terminal repeat (LTR)-directed gene expression (17, 122, 264). In the case of

HHV-6, this may be biologically relevant because HIV-1 and HHV-6 can productively infect the same types of cells and may productively coinfect human $CD4^+$ T lymphocytes (83, 313, 336). It is well documented that in vitro HHV-6 infection can activate the expression of heterologous genes linked to the HIV-1 LTR promoter (138, 157, 224). Other aspects of the in vitro interactions between HHV-6 and HIV-1 are discussed in the next section. Several genes and cloned DNA fragments can transactivate the HIV-1 LTR promoter. These include HHV-6A(U1102) ORFs DR7, U16, U27, and U94 (191, 299, 350, 382, 507, 511, 574); HHV-6A(GS) cloned fragments pZVB70, pZVB10, and pZVH14 (188, 225); and the HHV-6A(U1102) *Sal*I L fragment (257, 507). Differential transactivating properties were found for HHV-6A(GS) and HHV-6B(Z29) (225), with HHV-6A(GS) being able to transactivate the HIV-1 promoter in both stimulated and resting T cells and HHV-6B(Z29) being limited to transactivation in stimulated T cells.

One of the more interesting transactivating genes is U94, which encodes Rep_{H6}. Rep_{H6} appears to affect gene expression differently than does $Rep_{AAV\text{-}2}$. Rep_{H6} from HHV-6A(U1102) activates the HIV-1 LTR promoter in fibroblast cell lines but not in T cells, while $Rep_{AAV\text{-}2}$ inhibits HIV-1 LTR promoter activity in both fibroblasts and T-cell lines (511).

The function in the HHV-6 life cycle of most of the HHV-6 genes that can transactivate the HIV-1 LTR is not known; the transactivation of the HIV-1 LTR by these genes may be a general transcriptional effect.

Finally, superinfection with HHV-6A(GS) of cells that are latently infected with EBV can activate EBV lytic replication (168). This activation requires infectious virus and is mediated via a cyclic AMP-responsive element located within the EBV Zebra promoter (Zp). Zp controls the EBV gene product ZEBRA, which is responsible for disrupting EBV latency and initiating the lytic replication cascade. Interestingly, the HHV-6A gene product responsible for activating the Zebra promoter is not located within gene fragments known to *trans*-activate the HIV-1 LTR (167).

HHV-6 and HIV Interactions In Vitro

Many of the early HHV-6 isolates were obtained from HIV-1-infected persons. This, plus the fact that HHV-6 and HIV-1 infect overlapping subsets of $CD4^+$ lymphocytes, have led to the hypothesis that there is a specific interaction(s) between these viruses. Discussion of these interactions needs to take into account (i) interactions in simultaneously infected cells, (ii) the probability of simultaneous infections, (iii) specific interactions in the host that do not require the simultaneous presence of the viruses in the same cell, (iv) nonspecific effects of infection with one virus on the pathobiology of the other, and (v) the possibility of interactions relevant only in laboratory experiments.

As described above, HHV-6 can transcriptionally *trans*-activate the HIV-1 LTR. Other DNA viruses, such as HSV-1 (371, 372), HCMV (122), EBV (264), papovaviruses (190), and adenoviruses (376), are able to *trans*-activate HIV-1 LTR-directed gene expression in vitro by a *tat*-independent mechanism, but their interactions in vivo remain unclear. HHV-6 and HIV-1 can productively coinfect human $CD4^+$ T lymphocytes. In laboratory experiments, both stimulatory and inhibitory effects have been noted. In coinfections of lymphocytes with HIV-1 and HHV-6A(GS), dually infected lymphocytes were found, and accelerated HIV-1 gene expression and an enhanced cytopathic effect were noted (336). In other experiments, coinfection with other HHV-6A isolates suppressed HIV-1 replication, led to reduced reverse transcriptase levels, and reduced HIV-1 antigen expression (83, 312, 313, 324, 410). These results indicate that the viruses can interact with each other and that the specific direction of the interaction may be sensitive to the precise experimental conditions.

In addition to the effect that HHV-6 can have on HIV-1, HHV-6 replication can be enhanced by coinfection of PBMC with HIV-1 (83). HIV-1 Tat protein expression has been observed to both enhance and inhibit HHV-6 replication (138, 466).

EPIDEMIOLOGY

A number of factors may account for differences in reported HHV-6 seroprevalence. These include subjectivity in interpretation of immunofluorescence assays (IFA), differences in the assays and the isolate used as a source of the antigen, and the definition of cutoff values used for negative titers. In addition, there have been few attempts to distinguish between HHV-6A and HHV-6B in serologic studies, and there is significant cross-reactivity between both HHV-6 variants and HHV-7. Lastly, geographic variation of prevalence may play a role in determining the age of infection with HHV-6. In spite of these limitations, the accumulated information strongly points to infection with HHV-6 occurring early in life among widely distributed populations around the world.

Age

HHV-6 is normally acquired at a very early age. Reported rates of seroprevalence by age 13 months have been 64 to 83% in countries ranging from the United States to the United Kingdom, Japan, and Taiwan (64, 68, 229, 278, 392, 568). A large study of 2,427 young children in the United States who were examined for serologic and PCR positivity found that about 10% of infants less than 1 month of age were PCR positive, compared with 66% at 1 year (208). PCR positivity was thought to reflect past HHV-6 infection, with persistence of viral DNA in PBMC. The geometric mean antibody titer declined sharply from an age of less than 1 month to a nadir at 3 to 4 months, with a steep increase thereafter up to age 16 to 18 months. The rise in titer was delayed by about 2 months relative to the increase in PCR positivity. The data were interpreted as being consistent with a loss of maternal antibody (and protection from infection) in the early months after birth, with a high rate of infection occurring in succeeding months. Others have observed that rates of seroconversion are highest between 6 and 12 months of age (229). A number of investigators have reported a decline in seropositivity with age among adults (68, 156, 311, 383, 557), but others have found no significant differences (64, 392) or even an increase after age 62 (555).

Geographic Distribution

HHV-6 infection is widespread throughout the world. Among various populations, rates of seroprevalence have been reported to be as low as 20% among pregnant Moroccan women (423) to as high as 100% among asymptomatic Chinese adults (311). Between 60 and 90% of pregnant women in the countries of sub-Saharan Africa were found to be seropositive (423), while seroprevalence varied from approximately 39 to 80% among ethnically diverse adult populations from Tanzania, Malaysia, Thailand, and Brazil (40, 69, 159, 310, 319, 549). No significant ethnic differences were noted between native Brazilians and Japanese immigrants living in northeast Brazil (319). Several studies have observed modestly increased seroprevalence among females compared with males (64, 69, 106, 319), while another noted no difference in seroprevalence between genders (453). Reports from industrialized nations such

as the United States and Japan have described rates of seroprevalence from 72 to 95% among populations including children and adults (68, 392, 557).

HHV-6A and HHV-6B Variants

The initial description of HHV-6 reported the isolation of a virus, known as HHV-6A(GS), from a 17-year-old with acute lymphoblastic leukemia (ALL) (1, 450). This virus is representative of the HHV-6A variant, which has been obtained primarily from adults (3, 35, 144), most of whom were chronically ill. Most HHV-6 isolates from children, however, have been variant B, which is etiologically associated with ES and other pediatric febrile illnesses (see below) (131, 405, 455). As briefly mentioned in the section on nomenclature and classification, HHV-6B has also been isolated or detected in a number of other serious illnesses, which will be described in detail below. HHV-6A has yet to be definitively associated with any human disease. However, HHV-6A has been detected more frequently in two illnesses, KS (61) and chronic fatigue syndrome (139, 551), than in controls and has been found in association with other conditions, including AIDS (284, 450). Dewhurst et al. (131) enumerated the following possibilities for apparent differences in the epidemiology of HHV-6A and HHV-6B. (i) HHV-6A isolates have come from immunocompromised hosts or those with other diseases and hence may be unrepresentative of the population at large. (ii) There may be differences in the propensity for latency, reactivation, or sequelae between variants A and B. (iii) HHV-6A infection may occur later, in general, than HHV-6B infection. (iv) Primary infection with HHV-6A may be asymptomatic or cause nonfebrile illnesses. Understanding the differences in the epidemiology of HHV-6A and HHV-6B will require further studies.

Transmission

The question of how HHV-6 is transmitted from one person to another has not yet been fully answered, although it appears that transfer via saliva from mother to infant is the most common route. In several early reports, infectious HHV-6 was described as being present in saliva from nearly every person tested (211, 311, 409). After the discovery of HHV-7 and its unambiguous identification as a constitutive inhabitant of human saliva (55, 546), it was hypothesized that the salivary HHV-6 detected in the studies described above may have actually been HHV-7, the misidentification being due to cross-reactivity of some HHV-6 diagnostic reagents with the then-unidentified HHV-7 (57, 546). Upon reanalysis with HHV-6- and HHV-7-specific reagents, the saliva isolates originally described as HHV-6 by one group (211, 409) have been identified as HHV-7 (210). Nonetheless, several well-defined HHV-6B isolates have been obtained from saliva, but it is clear that HHV-6B is present in saliva at lower frequency than is HHV-7 (55, 312, 374), and no HHV-6A isolates from saliva have been described. In addition, HHV-6 DNA has been identified in saliva by PCR with HHV-6-specific primers (113, 136, 503). Estimates of the prevalence of HHV-6 DNA in saliva have varied widely, however. In one PCR-based study (113), salivary samples from 90% of persons examined contained HHV-6 DNA, while in another PCR-based study only 3% of persons were positive, although 63% of salivary gland biopsy specimens were positive (136). This finding is consistent with the possibility of salivary glands being a reservoir of persistent or latent viral infection. Detection rates can vary with age, peaking at 1 year of age and decreasing thereafter (503). Estimates of the quantity of viral DNA present in saliva have varied widely for inapparent reasons, ranging from 100 (243) to over 50,000 (113) copies of DNA per ml of saliva.

As noted above, maternal antibody may protect infants from infection early in life, but such protection is not universal. In the study by Hall et al. (208), 13% of infants with primary infection were younger than 2 months of age, including one only 14 days old. Infants who became infected in the first few months postpartum had lower mean antibody titers to HHV-6 than did others who were uninfected at a similar age. This observation suggests the possibility of intrauterine or perinatal transmission. This mode of transmission must be uncommon, however, since HHV-6-specific immunoglobulin M (IgM) was absent in serum from newborns (161) and present in only 2 of 799 samples of cord blood (149). HHV-6 DNA was not detected in throat swab specimens from any of 14 healthy neonates by PCR, suggesting the absence of oropharyngeal virus shortly after birth (265).

Nevertheless, several studies suggest that intrauterine or perinatal transmission can occur. One of 52 aborted fetuses of mothers infected with HIV-1 was positive for HHV-6 DNA, which was present in peripheral blood lymphocytes, thymus, liver, spleen, brain, and cerebrospinal fluid (CSF) (36). In two of three pregnant women who experienced spontaneous abortions and who had positive IgM antibody to HHV-6 in serum, virus-specific antigen was detected in abortive chorionic villous tissue, also consistent with intrauterine infections (36). The detection of HHV-6B DNA by PCR in the cervices of nearly 20% of women in late pregnancy, compared with 6% of nonpregnant controls, suggests reactivation of HHV-6 infection during pregnancy, with the possibility of perinatal transmission of the virus (390). Others also found HHV-6 DNA by PCR in the vaginal secretions of 10% of women visiting a sexually transmitted diseases clinic, consistent with the hypothesis of perinatal (or even sexual, on rare occasions) spread (303). A recent report of two cases of fulminant hepatitis associated with HHV-6B infection in neonates 3 and 5 days old is also suggestive of pre- or perinatal transmission (358). In both cases, HHV-6B isolated from the blood of mothers and infants exhibited DNA sequences characteristic to each mother-and-child pair, strongly arguing for pre- or perinatal transmission. Two other cases of presumed or apparent HHV-6 infection in the pre- and/or perinatal periods resulted in death or residual encephalopathy (537). However, the only evidence for HHV-6 infection in these cases was serologic, and other possible explanations for disease were not ruled out.

Saliva may act as a vehicle for transmission from mothers to infants. HHV-6 isolates obtained from the blood of several children who had ES and from the saliva of their healthy mothers yielded virtually identical restriction fragment profiles, although variation was apparent among isolates from different families (374). In another case, HHV-6 DNA was persistently detected by PCR in the saliva of a mother whose 6-month-old infant developed ES with HHV-6 viremia (482), but no strain analysis was done. PBMC of four mother-infant pairs were examined for HHV-6 DNA by PCR (523). In three of the four cases, mother and child had PCR products with identical DNA sequences. In one of these three pairs, however, the mother's saliva also contained HHV-6 that differed in its DNA sequence from the virus detected in her and the child's PBMC; this finding suggests that the mother harbored two distinct HHV-6 strains. For one infant-mother pair with identical PBMC PCR products, DNA sequence analysis indicated infection with HHV-6A, while in all other cases HHV-6B was detected. The recovery of HHV-6 DNA by PCR from throat swabs from a mother and of triplets who developed sequential ES, along with the recovery of HHV-6 with an identical re-

striction fragment pattern from the PBMC of all three children, is consistent with salivary transmission within a family (503). For other subjects in this study, the rate of HHV-6 PCR positivity in throat swab specimens rose to a peak of 87% for children aged 12 to 23 months and then declined to 32% for adults, suggesting that child-to-child transmission may occur. Such a possibility was also indicated by an outbreak of ES in an orphanage, for which isolated viruses had identical restriction enzyme patterns (389).

Two reports suggest the possibility of infection with different HHV-6 strains. In the first, 28 children were identified with either second ES episodes or ES which followed an afebrile exanthem (300). Many of these children either seroconverted to HHV-6 or had no rise in preexisting HHV-6 antibody titers in conjunction with the second illness, suggesting that one of the two exanthems in each case was caused by an agent other than HHV-6. However, significant rises in HHV-6 IgG levels occurred in five children who had preexisting HHV-6 IgG. For two of the patients, anti-HHV-6 IgM was present in both the acute- and convalescent-phase specimens. These results suggest that children who were already infected with HHV-6 may have been reinfected with a second strain of HHV-6 or that reactivation of a previously acquired strain can cause clinical illness. Infection with another herpesvirus with antigenic cross-reactivity to HHV-6 (e.g., HHV-7) may also account for these findings.

In the second report, an adult harbored HHV-6 strains in PBMC and saliva that differed from each other in their DNA sequences (523). In addition, variation was seen in the nucleotide sequence of HHV-6B detected in PBMC obtained sequentially over a period of 10 months from a child. The child had clinical illness coincident with the change in sequence. Given the genetic stability of herpesviruses, reinfection with a second strain was thought to be more likely than mutation. Among over 200 children from whom HHV-6 was isolated by this group of investigators, this was the only case of apparent reinfection in an infant with preexisting anti-HHV-6 antibody.

The potential for fecal-oral spread, expected primarily among children, is suggested by the observation that stool specimens can be persistently or intermittently positive for HHV-6 DNA by PCR for up to 6 months after an episode of ES (482). Such transmission has not been documented, however; given the epidemiology of HHV-6 infection, this is unlikely to be a major mode of transmission.

Breast-feeding has been proposed as a potential route of transmission of HHV-6, both during and after the perinatal period. However, HHV-6 DNA has not been detected in breast milk (150), and children who were not breast-fed seroconverted to HHV-6 during the same age range as did breast-fed children (498). Although intrauterine or perinatal transmission may occur infrequently, the epidemiologic data described above indicate that most HHV-6 infections occur later than these periods. Taken together, the epidemiologic investigations, the demonstration of HHV-6 in saliva, and the finding of identical viral restriction endonuclease fragment profiles and PCR products in specimens from mother-infant pairs support the hypothesis that the most common mode of infection with HHV-6 is by salivary transmission from mother to child.

CLINICAL MANIFESTATIONS

Primary Infection

Children. In the early 1950s, an infectious agent was identified as the etiologic agent of the common childhood disease ES (also known as roseola infantum or sixth disease) through demonstration of human-to-human (214, 261) and human-to-primate (261) transmission. The ethical issues surrounding the human-to-human transmission experiments were recently discussed by Griffiths (204). Nearly four decades passed before Yamanishi et al. identified the virus that was eventually shown to be HHV-6B as the infectious agent responsible for most ES cases (556). Since then, HHV-6B also has been identified as the causative agent of other related primary illnesses in young children (131, 208, 412, 417, 455). Primary HHV-7 infections have been associated with a minority of ES cases (502, 515), although infection with this virus appears to occur later than that with HHV-6B (59, 503, 547).

The classic presentation of ES commences with a fever that may exceed 40°C and usually lasts from 3 to 5 days (30, 63, 297). As temperature normalizes, a macular or maculopapular rash develops, generally beginning on the trunk, with later spread to the extremities and often to the neck and face. Typical dermal lesions are 2 to 3 mm in diameter and fade with pressure (297). In one study of Japanese children, about half had a morbilliform rash while most of the remainder had a rubella-like rash (26). Cases clinically identified as either measles or rubella can be due to primary HHV-6 infection (54, 494). Cervical, postauricular, and/or occipital lymphadenopathy are also common physical findings.

Additional findings in another study of Japanese children (30) included diarrhea (68% of patients), Nagayama's spots of the soft palate and uvula (65%), cough (50%), edematous eyelids (30%), bulging fontanelle (26%), nonspecific prodrome (14%), and febrile convulsions (8%). Lymphocytosis and neutropenia are often observed when the rash is present (41) but not to neutrophil levels indicative of immunosuppression. In one study, approximately 60% of Japanese children experienced clinically defined classic ES (299), although only about 30% of children in the United States do (297). Whether this difference is the result of differences between HHV-6 strains, host genetic factors, frequency of medical consultation for mild childhood illness, or environmental factors is unknown. Even though the classic form of the illness may include such manifestations as bulging fontanelle and febrile seizures, ES normally does not require specific treatment and usually resolves without long-term sequelae.

HHV-6 can be cultured easily from PBMC obtained from patients during the febrile phase of ES (26, 27, 387, 556), and HHV-6 DNA can be detected in the plasma or serum of children with ES (459). In patients with longer febrile periods, increased numbers of infected cells in the blood are observed within 3 to 4 days of onset. After 5 days of the classic illness, viremia has generally resolved (10, 26, 27). The disappearance of viremia correlates with the appearance of HHV-6 neutralizing antibody (27, 519, 570).

Early case reports described some patients with primary HHV-6 infections presenting with fever without rash (485) and rash without fever (27). Several recent publications have described infectious mononucleosis-like illnesses in association with HHV-6 seroconversion in young children (209, 255, 524). A 1-year-old child from Israel experienced such an illness with hepatosplenomegaly and lymphocytosis including 65% atypical cells (209). This child also had a diffuse, confluent rash covering nearly the entire body. Another patient with primary infection had a peripheral blood smear demonstrating 6% atypical lymphocytes and 3% blast forms, prompting bone marrow examination to rule out leukemia (524). This child had fever and hepatomegaly and experienced a complex seizure; no dermatologic abnormalities were reported. A young child from Japan was observed to have an otherwise typical case of ES in which vesicular skin lesions, similar to those of varicella, were

TABLE 3. Signs and symptoms in febrile children according to HHV-6 culture status[a]

Sign or symptom	No. (%) of children:		P
	HHV-6 positive (n = 34)	HHV-6 negative (n = 209)	
Fever ≥ 40°C	22 (65)	90 (43)	0.002
Malaise, irritability	28 (82)	121 (58)	0.02
Inflamed tympanic membranes	21 (62)	67 (32)	0.002
Nasal congestion	19 (56)	84 (40)	0.09
Cough	9 (27)	67 (32)	0.6
Rhonchi, wheezing or crackles	8 (24)	36 (17)	0.41
Diarrhea	10 (29)	84 (40)	0.24
Vomiting	7 (21)	96 (46)	0.02
Rash	6 (18)	25 (12)	0.34
Seizure	1 (3)	16 (7.6)	0.58

[a] Reprinted from reference 208 with permission of the publisher.

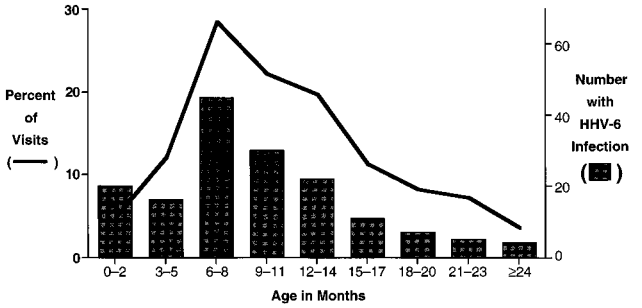

FIG. 4. Number of children presenting to the emergency department with illness from primary HHV-6 infection (bars) and visits due to such infections as a percentage of all emergency department visits for acute febrile illnesses (curve), according to age. Reprinted from reference 208 with permission of the publisher. Courtesy of Caroline B. Hall.

found, and HHV-6 DNA was detected in the lesions by PCR (561). Additional recent investigations (54, 208, 228, 412, 416, 417, 460, 494) have confirmed that many HHV-6 primary infections do not present clinically as classic ES. Of 243 febrile children 2 years of age or younger who presented to the emergency department of a large academic hospital in the United States, 34 (14%) had HHV-6 viremia (417). High temperature (mean, 39.7°C), malaise and irritability, and tympanic membrane inflammation were noted most often (Table 3). Other findings included respiratory and gastrointestinal abnormalities and concurrent rash. The incidence of these latter signs and symptoms was not significantly different between the HHV-6-positive and -negative groups, with the exception of vomiting, which was seen more often in patients from whom HHV-6 was not recovered. Only 3 (9%) of the 34 children developed a rash following resolution of fever as in classic ES. One case of febrile seizures was observed. This study demonstrated the lack of characteristic ES among HHV-6-infected children in the United States who were sufficiently ill to warrant emergency evaluation. A similar conclusion was drawn from a study of young children in Italy (412).

In a follow-up study in the United States, among children 3 years of age or younger who presented with acute febrile illness, 160 (9.7%) of 1,653 children had primary HHV-6 infection (208). The youngest was 2 months old, and all were less than 26 months old. The peak age of acquisition was 6 to 9 months, with 45 (24%) of 186 visits to the emergency department apparently being due to primary HHV-6 infection (Fig. 4). Of the 160 HHV-6-positive children, 21 (13%) required hospitalization, with 65% of these children having suspected sepsis and 15% having diarrhea and dehydration. Of 160, 21 (13%) experienced seizures and only 27 (17%) were eventually diagnosed with ES. Although 41% of those with primary HHV-6 infection had upper respiratory tract signs, only 2 (<1%) of the 160 patients were hospitalized with lower respiratory tract disease. Respiratory syncytial virus was isolated from one of the infants. These data suggest that more severe respiratory disease is a rare complication of primary HHV-6 infection in children. Similar results were obtained in another study of children with abnormalities of the respiratory tract associated with acute HHV-6 infection (538). HHV-6 genomes were present in the PBMC in 37 (66%) of 56 children studied by PCR 1 to 2 years after primary infection. Over that period, HHV-6 antibody titers increased in 30 (16%) of 187 children, and 17 (6%) of 278 children showed fluctuation of PCR results from positive to negative to positive, suggestive of reactivation of persistent viral infection. This study demonstrated the potential for serious complications among young children with primary HHV-6B infection.

The larger investigations described above probably overestimate the proportion of children acutely infected with HHV-6 who have severe manifestations or complications, since these children were sick enough to require medical attention. The most severely ill children may not represent the vast majority of children who have primary HHV-6 infection. Nonetheless, additional case reports illuminate the spectrum of disease which may be observed in children with primary HHV-6 infection. These include cases of liver dysfunction (495, 500), hepatosplenomegaly (228, 236), fatal (358) and nonfatal (32, 358) fulminant hepatitis, intussusception (29), thrombocytopenia (228), thrombocytopenic purpura (275, 445, 446, 563), fatal (96, 230) and nonfatal (96, 488) hemophagocytic syndrome, and disseminated disease resulting in death (415).

Adults. As shown by epidemiologic studies, most persons are infected with HHV-6 at an early age. Consequently, primary infection of adults is relatively rare. Such infections appear to have greater severity than do most primary infections in children. Mononucleosis-like syndrome (13, 193, 476), prolonged lymphadenopathy (237, 383), and fulminant hepatitis (468) have been described. Several patients with features of heterophile-negative mononucleosis also had erythroderma (13, 193, 489), and in one of these, HHV-6 antigens and nucleic acids were found in lymphocytes infiltrating the skin (489).

A previously healthy young woman presented with fever, generalized skin rash, jaundice, widespread lymphadenopathy and leukocytosis with 45% atypical cells. Early in the course of illness, she had positive serum IgG to HHV-6, EBV, and HCMV (titers from 1:10 to 1:40) and negative IgM to these same viruses. Serologic tests for human T-cell leukemia virus type 1 and hepatitis B virus were negative. Many cells in a lymph node biopsy were HHV-6 DNA positive by in situ hybridization (ISH), but DNA of EBV or HCMV was undetectable by this method. This patient's illness, which eventually resolved, was most probably due to primary HHV-6 infection, despite the lack of detectable anti-HHV-6 IgM (490).

A possible role for HHV-6 in the development of histiocytic necrotizing lymphadenitis (HNL; Kikuchi-Fujimoto disease) has been investigated on the basis of clinical manifestations similar to those in patients with HHV-6-associated lymphadenopathy (221, 383). However, no significant differences were found in the frequency of detection of HHV-6-specific antigens or DNA in tissues of HNL or non-HNL lymphadenopathy

(154, 491). Histiocytes but not T cells harbored HHV-6 in both HNL and non-HNL patients (491). Another recent investigation of HNL found no lymph nodes positive for HHV-6 DNA by PCR from among 20 patients examined (221).

An uncommon disorder which affects children and young adults is sinus histiocytosis with massive lymphadenopathy (SHML), or Rosai-Dorfman disease (172, 438, 439). SHML usually is characterized by painless cervical lymphadenopathy, occurring in exacerbations and remissions. This usually benign disorder is thought to be due to an unusual immunologic response to infection. One investigation by in situ hybridization found HHV-6 DNA in involved tissues from 7 of 9 SHML patients (309). In one patient, both HHV-6 and EBV DNAs were detected; in another, only EBV DNA was detected; and in a third, no DNA from either virus was detected. These results suggest that HHV-6 infection may induce some cases of SHML.

Central Nervous System Disease

As mentioned above, HHV-6 can infect cells of neural origin in vitro and tissues of the CNS in vivo, suggesting that the virus(es) may play a significant role in diseases of the brain or spinal cord.

In vitro studies. In cell culture, the Gambian HHV-6 isolate AJ forms syncytia with the embryonic glial cell line HEB, with immunofluorescent staining observed in shed cells (504). HHV-6A(GS) abortively infects the HTB-14 glioblastoma cell line, with loss of antigen-positive cells by day 10 postinfection; infected cells become enlarged and refractile. HHV-6B(SF) grows persistently at low levels in the neuroblastoma cell line SK-N-MC (312). Primary human fetal astrocytes can be infected by both HHV-6A and HHV-6B in cell culture, with formation of giant syncytia (213). There have been no reports of in vitro infection of primary neurons or oligodendrocytes by HHV-6, although evidence for infection of these cell types in vivo is described below.

Acute disease. Early descriptions of ES included complications such as febrile seizures, bulging of the anterior fontanelle, hemiplegia, meningoencephalitis, and residual encephalopathy (51, 75, 366, 558). Along with the recognition that HHV-6B is the major etiologic agent of ES, there have been reports of pediatric CNS involvement in the illness (26, 208, 228, 239, 249, 274, 289, 483, 565). The large study by Hall et al. (208) found that 21 (13%) of 160 HHV-6-infected children experienced febrile seizures, compared with 9% of those with non-HHV-6 febrile illnesses; the difference was not statistically significant. Seizures occurred after the first day of the illness in 9 (43%) of the 21 HHV-6-infected patients. Of children 12 to 15 months of age, 36% experienced febrile convulsions compared with only 13% of similarly aged children with non-HHV-6-associated illnesses. The difference in this age group did reach statistical significance. In this study, 7 (24%) of 29 CSF specimens were positive for HHV-6 by PCR, including 2 of 7 from children with convulsions. No sample contained HHV-6 that was infectious in cell culture.

HHV-6 was cultured from PBMC of 8 (19%) of 42 children experiencing a first febrile seizure (47). An additional three children seroconverted to HHV-6, such that 26% of the children had an initial febrile seizure thought to be associated with primary HHV-6 infection. This percentage is comparable to an estimated 31% of children in the study by Hall et al. (208).

The CSF of 172 children with evidence of acute or past HHV-6 infection was evaluated by PCR for the presence of HHV-6 DNA (84). Of these children, 72 (42%) had HHV-6 DNA present in CSF, of whom 7 had evidence for acute HHV-6 infection. Of the 142 children with evidence for past HHV-6 infection documented by HHV-6 DNA in PBMC, CSF, or both, 41 (29%) had HHV-6 DNA present in CSF only, suggesting that the CNS may be a site of viral persistence or latency. Other reports have also described the detection of HHV-6 DNA in the CSF of children with past primary or acute HHV-6 infection (208, 289, 483, 565).

The experience with the pediatric population suggests that HHV-6 may directly invade the CNS during primary infection. Cases of HHV-6-associated encephalitis in young children support this hypothesis (28, 239, 285, 354, 355, 558, 565), and those cases in adults are consistent with HHV-6 reinfection or reactivation in the CNS (355). A recent retrospective study of 138 patients is of particular interest in this regard (355). One group of 37 patients had previously undergone open brain biopsy for evaluation of encephalitis, while a second group of 101 patients with encephalitis had undergone prior PCR testing of CSF for HSV, the most common cause of sporadic viral encephalitis (536). PCR results for HSV were negative in all cases. Of the 138 patients, 9 were positive for HHV-6 in CSF by PCR with HHV-6-specific primers. In the two patients evaluated, variant B was identified. Two of the nine were children, ages 13 and 15 months, who may have had primary infection; however, no sera were available for analysis. Specimens from other patients were all seropositive, without significant differences between acute- and convalescent-phase samples. No significant differences were found in the clinical findings, laboratory test results, CNS imaging, or electroencephalographic studies between the 9 patients with HHV-6-associated cases and the 129 without evidence for HHV-6 involvement. Clinical outcome in the nine HHV-6-positive patients included full recovery in four, mild to modest residual neurologic impairment in three, a persistent seizure disorder in one, and early death in one. At autopsy, white matter necrosis and cortical myelin loss were observed in the patient who died. The authors of this publication concluded that HHV-6 is associated with encephalitis of variable severity and that variant B is involved in some cases.

In another recent investigation, 10 (20%) of 50 immunocompetent patients with meningitis or encephalitis had increased intrathecal anti-HHV-6 early antigen (EA; p41/38) IgM or IgG while none of 50 healthy controls did (403). Intrathecal antibody levels to 26 other viruses, including HSV, EBV, HCMV, VZV, arboviruses, echoviruses, and measles and mumps viruses, were not elevated in these patients. These results are consistent with active HHV-6 infection of the CNS in some cases of meningitis or encephalitis.

Direct evidence for HHV-6 infection of the CNS is provided by the case of a 14-month-old girl who died of fulminant encephalitis (285). This child presented with a brief history of fever, lethargy, cough, and seizures. Upon hospitalization, the patient was found to have a mild lymphocytosis, generalized lymphadenopathy, mild hepatomegaly, and hyperventilation with a pattern of interstitial pneumonitis on chest X-ray. A computed tomographic scan of the brain showed bilateral areas of decreased density. She was found to be HIV-1 seropositive and experienced a precipitous decline in the level of $CD4^+$ T lymphocytes. She died on day 5 after admission. Autopsy samples of the cerebral cortex demonstrated HHV-6 DNA, predominantly of variant A, by PCR. Immunohistochemical (IHC) staining with rabbit anti-HHV-6 hyperimmune serum demonstrated HHV-6-infected cells in gray matter, mostly in astrocytes but also in some oligodendrocytes and neurons. HHV-6B-specific MAb detected fewer HHV-6-infected cells, whereas IHC staining with probes for HIV p24 antigen, HCMV, HSV, VZV, and JC virus were all negative. ISH for EBV RNA was also negative. Although no HHV-6

serologic test results for this patient were available, the clinical course and laboratory findings suggest acute, overwhelming HHV-6A infection with development of fulminant encephalitis in an immunocompromised host.

HHV-6 invasion of the CNS was also shown in a second case of encephalitis which involved a 37-year-old BMT recipient who died 5 months after an allogenic transplant (148). IHC and nucleic acid hybridization studies of affected brain tissue demonstrated HHV-6 infection of astrocytes in white matter lesions, while neurons were involved in areas of affected gray matter. Demyelination was observed in regions of white matter involvement. Control studies with probes for HIV, HCMV, HSV, VZV, EBV, JC virus, and measles virus were all negative. Variant-specific probes indicated infection with HHV-6B.

CNS invasion in other conditions. Several publications have described the presence of HHV-6 nucleic acids or antigens in CSF or tissues of the CNS in clinical states other than primary infection or acute encephalitis. A report of PCR studies of brain tissue from immunocompetent persons dying of causes unrelated to HHV-6 infection noted that HHV-6 DNA was detected in 11 of 13 adults (variant A in the five positive cases examined), suggesting that many healthy persons silently harbor the virus or its DNA in the CNS (329). A follow-up investigation (328) found that six of nine immunocompetent adults who died of noninflammatory neurologic disease or illness unrelated to the CNS had HHV-6 DNA in brain tissue by PCR. Gray matter brain samples from four of seven AIDS patients were also positive for HHV-6 DNA. When primary brain tumor biopsy specimens were examined in another group of patients, only 6 (16%) of 37 (and only 1 [3%] of 30 without histologic evidence of nonneoplastic tissue) were positive, suggesting that HHV-6 is not a major pathogen in primary CNS neoplasms. The HHV-6 variant was not determined in this study.

Several other investigations have looked for evidence of HHV-6 involvement of the CNS in persons dying of AIDS, both in children and adults. A study of postmortem brain tissues by Saito et al. (448) obtained from children with progressive AIDS encephalopathy found that samples from four of five patients had HHV-6 nucleic acids demonstrable by ISH, primarily in the white matter oligodendrocytes but also in scattered astrocytes, macrophages/microglia, and neurons. In two patients whose samples were evaluable by nested PCR and restriction enzyme analysis, HHV-6B nucleic acids were identified. Whether the single patient with negative results had even been infected with HHV-6 was not determined. IHC studies, using anti-HHV-6 rabbit hyperimmune serum and MAb directed against various HHV-6 antigens, failed to detect HHV-6 protein products, although HIV-1 p24 antigen was found in all patients. As noted by the authors, such results are suggestive of latent HHV-6 infection of the CNS in patients with advanced AIDS encephalopathy. For two patients, infrequent coinfection of macrophages by HIV-1 and HHV-6 was observed. These authors concluded that HHV-6 may be an important opportunistic pathogen in pediatric AIDS patients.

Four of six unselected adults who died of AIDS had demyelinating lesions in brain tissue at the time of death (283). IHC staining with rabbit anti-HHV-6 hyperimmune sera and an HHV-6B-specific MAb demonstrated HHV-6-infected cells in areas of demyelination but not in histologically normal regions. In three of the patients, demyelination was restricted to a few foci, while in the fourth it was diffusely distributed. For this last patient, antibody probes to HCMV, HSV, VZV, JC virus, measles virus, and HIV p24 antigen did not detect the presence of these viruses, and EBV RNA was not found with an oligonucleotide probe. The neuropathologic findings in these patients were similar to those in the case of the BMT patient with encephalitis described above (148).

Levels of intrathecal anti-HHV-6 EA IgM and/or IgG were elevated in 30 (60%) of 50 patients with HIV encephalopathy (403). Normal levels for comparison were determined with CSF from 50 healthy controls. Intrathecal antibody levels to HSV, EBV, or HCMV were not correspondingly increased. The results for HCMV are curious, considering its frequent association with HIV encephalopathy (104, 456, 525, 542). This study suggests active HHV-6 infection of the CNS in many patients with HIV encephalopathy.

Achim et al. (7) examined autopsy brain tissue specimens, including cortical gray matter, cerebral white matter, and basal ganglia, from 45 AIDS patients with and without encephalitis. The acuteness of the encephalopathy and age of the patients were not specified, although most patients were presumably adults. HIV-1 and HCMV DNA were detectable by PCR in these tissue samples in approximately 75% and over 50% of patients, respectively. HHV-6 DNA was found only in the basal ganglia of one patient by a similar method.

Studies of HHV-6 CNS infection in persons with AIDS have not yielded entirely concordant results, at least on first glance. First, while several groups (283, 330, 403, 448) have reported evidence for the frequent presence of HHV-6 in the CNS of AIDS patients, others noted its rarity (7). Differences in techniques between this last study and the other investigations involving PCR (330, 448) may account for some of the differences in the results. The primers used in the work by Achim et al. (7), however, have been successfully used by others to identify HHV-6 DNA in PBMC (417). The studies that showed high frequencies of HHV-6 CNS infection all involved far fewer patients than studied by Achim et al., which may have biased the results. In addressing the differences in frequency of HHV-6 detection in the CNS in the study of adult AIDS patients by Achim et al., and their own pediatric study, Saito et al. (448) noted that HHV-6 may be more extensively disseminated in the CNS of children than adults because of the following: (i) primary HHV-6 infection in children in contrast to reactivation in adults, (ii) persistent infection of the CNS by HIV-1 (presumably of greater duration in adults), and (iii) preexisting immunodeficiency at the time of HHV-6 infection (in children). These possible effects notwithstanding, it is surprising that the Achim et al. study found so little evidence for the presence of HHV-6 in the CNS, given the results of previously described investigations involving healthy persons and those with AIDS, despite the small numbers of subjects for the latter. An additional report (discussed below) also found evidence for HHV-6 DNA by PCR in the brains of a large majority of over 80 healthy and ill persons, again supporting the concept of CNS invasion of HHV-6 in most persons. Further work is necessary to determine if adults with AIDS differ substantially from other groups with respect to the presence of HHV-6 in the CNS. At present, the available data indicate that most persons harbor HHV-6 in the CNS.

Second, several studies of HHV-6 expression in the brains of AIDS patients have shown apparent differences. In the pediatric study by Saito et al., no evidence was found for HHV-6 gene expression, while the investigation by Knox and Carrigan (283) found HHV-6 antigens in areas of demyelination. One possibility is that HHV-6 expression in the CNS of persons with AIDS differs between children and adults, possibly related to primary versus reactivated infection. Another potential explanation involves tissue sampling effects. The work of Knox and Carrigan found only a few foci of affected brain in three of the four patients, while other areas appeared normal. These histologically normal regions might contain latent HHV-6, as

was found in the pediatric study. The small patient numbers in both of these investigations may also have affected the conclusions. The publication describing the presence of intrathecal HHV-6 antibody (403) in a majority of those with AIDS encephalopathy supports active HHV-6 infection of the CNS in AIDS patients, even if only in a few localized regions of the brain.

Studies of HIV-infected persons have yielded important findings with respect to HHV-6 and the CNS. They have also indicated how much remains to be learned about interrelationships between HHV-6, the CNS, and AIDS.

HHV-6 invasion of the spinal cord was demonstrated in the case of an elderly woman who developed a chronic myelopathy with progressive spastic paraparesis (345). At autopsy, 2 years after the onset of symptoms, there was widespread demyelination, axonal loss, chronic inflammation, and gliosis. IHC studies with a polyclonal anti-HHV-6 antibody showed reactivity predominantly with astrocytes in regions of white matter degeneration. Staining with HSV-1, HSV-2, HCMV, and EBV antibodies was negative. DNA extracted from abnormal spinal cord tissue yielded a PCR amplification product with HHV-6-specific primers. PCR results for HSV, VZV, HCMV, human T-cell leukemia virus type 1, and HIV were negative. Although causation was not proven, these findings suggest a possible role for HHV-6 in the pathogenesis of some cases of chronic myelopathy.

Multiple sclerosis. Among the most interesting reports of a role for HHV-6 in the pathogenesis of serious illness are those indicating a possible association of HHV-6 with MS. This disease is characterized clinically by neurologic signs and symptoms which wax and wane over time and involve multiple nerve pathways. Young adults living in temperate climates are most often affected. In northern and central Europe, southern Canada, and the northern United States, MS prevalence is at least 60 cases per 100,000 persons (177). Pathologically, plaques of demyelination of neuronal axons are observed that most often involve the optic nerve, periventricular white matter, cerebellum, brain stem, and spinal cord. The leading hypothesis of the pathogenesis of this disease is that an autoimmune, inflammatory reaction results in the destruction of myelin, which is composed of oligodendrocyte cell membranes (272, 477). Viruses have long been suspected as etiologic agents which trigger an autoimmune response. Indeed, a viral etiology has been established for four human demyelinating diseases (248). These include progressive multifocal leukencephalopathy (JC virus), postinfectious encephalomyelitis (measles virus), and HIV encephalopathy and myelopathy. Animal models of demyelinating diseases due to viral infection also exist (248). Demonstrating a viral etiologic agent of MS has been more difficult, with many proposed candidates, ranging from measles and influenza viruses to herpesviruses and a retrovirus (248).

Higher average antibody titers to HHV-6 occur in MS patients than in controls (469, 540). HHV-6 DNA has also been detected by PCR in the CSF of MS patients but not in that of matched controls (540). A very intriguing recent report by Challoner et al. (87) described the expression of HHV-6 in oligodendrocytes near plaques in brain tissue of MS patients. This investigation involved searching for potential pathogens in MS brain tissue by using representational difference analysis, a technique which employs successive rounds of subtractive hybridization and PCR amplification to enrich for DNA sequences present in abnormal tissue (320). Using this technique, these investigators found a DNA sequence in the brains of MS patients that was nearly identical to a portion of the gene that encodes the HHV-6B homolog of the conserved herpesvirus MDBP. HHV-6 was detected by PCR in over 70% of 86 brains from MS patients and controls, consistent with the high prevalence of HHV-6 in the CNS, as described above. The vast majority of viruses found were HHV-6B. In contrast, a search for other viral pathogens yielded few positive results. IHC studies with MAb to two HHV-6 proteins demonstrated nuclear staining of white matter oligodendrocytes in 12 (80%) of 15 MS patients but in none of 45 individuals without MS, a difference that was highly statistically significant (Fig. 5). Nuclear staining was observed more frequently near plaques in MS patients than in uninvolved white matter. Prominent cytoplasmic staining of neurons occurred in gray matter around plaques in MS patients (this was also noted in some controls). Of interest, positive nuclear staining of oligodendrocytes in areas without any demyelination or inflammation occurred in four MS patients. These data are consistent with viral expression preceding inflammatory changes within the local tissue, rather than occurring as a consequence of inflammation. Consistent with this finding, several control patients had an inflammatory process such as encephalitis, yet no oligodendrocyte staining for HHV-6 was observed in them.

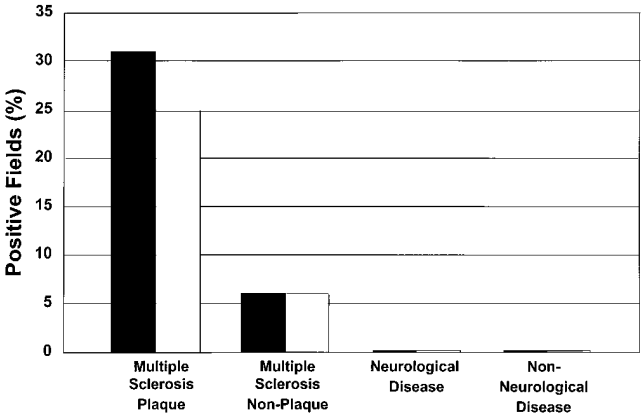

FIG. 5. Quantitation of 101K nuclear staining of oligodendrocytes. The specimens included 50 plaques and 73 regions of normal white matter from 13 MS patients and 83 white matter regions from 13 controls. Each region contained at least 2,000 oligodendrocytes. Solid bars denote fields with 1 to 10% stained oligodendrocytes; empty bars denote fields with 11 to 50% stained oligodendrocytes. Adapted from reference 87 with permission of the publisher.

One interpretation of this study, which used MAb to two structural proteins of HHV-6, is that low-level viral replication in persistently infected CNS tissue leads to a local inflammatory response in certain susceptible individuals, causing demyelinating lesions of MS. The observations of differences in IHC staining between MS patients and individuals without MS and between involved and uninvolved areas in the brains of MS patients are consistent with this hypothesis. An alternative explanation, i.e., that the IHC staining was nonspecific, appears unlikely, given the negative results in individuals without MS, including some with inflammatory diseases, and the use of two well-characterized MAb that recognize different virally specified proteins. The finding of PCR-positive results for HHV-6 and the lack of these results for other viruses in CNS tissue also enhances confidence in the specificity of the IHC staining. It is also possible that replicating virus could merely be an innocent bystander in areas of disease, although the data indicating viral expression prior to inflammatory changes argue against this. An additional possibility is that the HHV-6 antigens are present due to postmortem viral replication specifically in areas of MS lesions; this appears remote and is unprecedented.

In a subsequent retrospective case study, CNS tissues from a young woman who died of a rapidly progressive demyelinating disease were examined by IHC staining with an HHV-6-specific antiserum (80). The disease was diagnosed as acute MS both clinically and histologically postmortem. Mononuclear cell infiltration was widespread in CNS tissues. HHV-6-positive cells were present within and near many areas of demyelination. HHV-6-infected cells were not detected in tissue specimens lacking evidence of demyelination or with older-appearing plaques. The types of cells harboring HHV-6 were not unambiguously determined but were thought to include oligodendrocytes, astrocytes, and microglial cells. The authors noted that this patient's course was more fulminant that in most cases of MS and suggested that her case may be pathogenically distinct from more typical MS. They hypothesized that HHV-6-associated demyelination may involve direct cytolysis of glial cells as well as virus-mediated induction of cytokines, such as TNF-α, that might harm myelin (462).

The number of MS cases examined by IHC in these two studies was small, and the results are far from definitive in proving an etiologic link between HHV-6 and MS. Nevertheless, the findings are exciting and are certain to spark further investigation of the association between HHV-6 and this relatively common acquired neurologic disease.

Disease in Immunocompromised Hosts

Persons with compromised immune systems due to either natural means or pharmacologic immunosuppression are among those most at risk for disease caused by herpesviruses. Such illness may be due to primary viral infection or reactivation of latent or persistent infection. Possible associations of HHV-6 and disease in immunocompromised hosts have been sought since the discovery of the virus.

Renal transplants. Organ transplant recipients have been among those most closely examined for possible links of HHV-6 to serious illness. Of 21 renal allograft recipients, the 8 who had significant posttransplantation rises in HHV-6 antibody titer also developed severe rejection of the transplanted kidney (388). Of note, all eight had received either anti-CD3 MAb (OKT3) or antilymphocyte globulin (ALG) part of their rejection treatment protocol. Of the other 13 recipients without a significant HHV-6 antibody titer increase, only 5 (38%) experienced rejection, and 2 of these 5 received either OKT3 or ALG. Biopsy specimens were obtained from rejected kidneys of nine other patients, and viral antigen was identified by immunofluorescence in five specimens in tubular epithelial cells, infiltrating histiocytes, and lymphocytes. Another study reported serologic evidence for primary HHV-6 infection or a significant rise in antibody titer to HHV-6 in two-thirds of 53 allograft recipients (359). However, no correlation with rejection was found. A third investigation observed either HHV-6 viremia or significant HHV-6 antibody titer increases in 36 (55%) of 65 patients in the first 3 months after transplantation (566). There was no correlation with rejection. Neither OKT3 nor ALG was used for treatment of rejection in the studies in which no correlation of HHV-6 with rejection was found, although in one of them (566), short-term ALG was administered to some patients as rejection prophylaxis; no correlation with rejection was noted. No details were provided about ALG or anti-CD3 dosing for either study. Another potential complication is that possible contributions of cross-reacting HHV-7 antibodies to the measured anti-HHV-6 titers cannot be excluded (see the section on immune response, below). HHV-6 replication in vitro can be stimulated by antibodies to CD3 (269, 435). Consistent with the hypothesis that HHV-6 may be associated with renal allograft rejection in patients treated with OKT3 or ALG is a recent report of a transplant recipient who experienced rejection and was treated with OKT3 (242). The HHV-6 IgM level was elevated but there was no evidence of HCMV reactivation in a patient known to be previously infected with both viruses. Sustained improvement in transplant function was not achieved until the patient was treated with ganciclovir, which has shown antiviral activity against HHV-6 in vitro.

Two other recent publications regarding renal transplant recipients are of interest. A study of 16 transplant patients in Turkey found that 10 (63%) had HHV-6 DNA detectable by PCR in PBMC, compared with 7 (44%) of 16 healthy controls (550). Of the 10 transplant recipients, 7 (70%) had variant B while 3 (30%) had variant A. HHV-6 DNA was detected in four (80%) of the five transplant patients who experienced rejection. The B variant was found in the two patients with acute rejection and in three of the four who were positive for HHV-6 DNA. HHV-7 DNA was found in only 3 (19%) of the 16 transplant recipients and in only 1 of those experiencing rejection. That patient, with chronic rejection, also had HHV-6B detected. Whether the treatment for individual patients experiencing rejection involved OKT3 was unclear.

In the largest investigation to date involving retrospective IHC examination of renal allograft specimens, HHV-6 structural proteins were detected in 63 (61%) of 105 biopsy samples from 76 renal transplant recipients (226). Viral antigens were observed in distal tubular epithelial cells and in rare lymphocytes infiltrating the interstitium, confirming previous observations (298, 388). HHV-6 antigens were present in 28 (74%) of 38, 3 (75%) of 4, and 8 (73%) of 11 specimens from patients experiencing acute or accelerated rejection, or cyclosporine nephropathy, respectively (226). In addition, HHV-6 antigens were identified in only 11 (46%) of 24 samples from transplant recipients with chronic rejection. The difference in HHV-6 antigen prevalence between acute and chronic rejection was statistically significant.

Bone marrow transplants. (i) Clinical associations. The organ transplant population most intensively evaluated for HHV-6 involvement in posttransplantation disease has been BMT recipients. HHV-6 activity has been associated with rash and graft versus host disease (GVHD), pneumonitis, sinusitis, febrile episodes, and suppression of graft outgrowth. Although it is difficult to prove an etiologic association of the virus with these disease events, their propinquity with HHV-6 activity in the absence of other possible causes suggests that at the very least a subset of these events is due to HHV-6 activity. The observations are detailed below.

An early study of the association of HHV-6 activity with BMT found that 10 (40%) of 25 pediatric BMT recipients had infectious HHV-6 in PBMC or bone marrow mononuclear cells (567). Virus was cultured between days 14 and 22 posttransplantation. Two additional patients had significant increases in their HHV-6 serologic titer, so that 12 (48%) of the 25 patients showed evidence of HHV-6 infection. Of the 10 who had positive HHV-6 cultures, 4 developed a skin rash resembling GVHD and 3 of these were febrile when the rash appeared. In one of these patients, GVHD was confirmed histologically. None of the 13 patients without evidence for HHV-6 infection developed a skin rash, suggesting that HHV-6 is associated with dermal eruptions in young BMT recipients. As with the renal transplant studies described above, this investigation did not rule out the possibility that some of the HHV-6 serologic titer measurements were affected by cross-reacting antibodies to HHV-7.

Several other reports have described HHV-6-associated der-

mal manifestations in BMT recipients. A recent case report summarized the exanthems observed in an infant girl who underwent three successive BMTs for severe combined immunodeficiency (SCID) (363). After the third transplant, biopsy-proven GVHD occurred, with skin and liver involvement. Long-term steroid therapy resulted in resolution of the histologic features of GVHD, although the rash did not fully resolve. About a year later, two new rashes developed successively, with the latter including widespread papular lesions, which demonstrated chronic dermatitis without GVHD on biopsy. DNA from the tissue sample was positive for HHV-6 by PCR and blot hybridization. Negative results were obtained for other herpesviruses and parvovirus B19. The patient, who was HHV-6 seropositive prior to transplantation, had HHV-6 IgM before and shortly after the later rashes. The authors concluded that the patient had a chronic, active HHV-6 infection with skin involvement. Another study found exanthematous papular rashes in six BMT recipients treated for SCID or osteopetrosis (23). Skin biopsy specimens of all six were positive for HHV-6 DNA by PCR. Histopathologic analysis showed dermatitis in the two patients with osteopetrosis and GVHD in the four BMT recipients with SCID. These investigators suggested that HHV-6 may cause GVHD and other exanthems in such immunosuppressed hosts. A third study (described more fully below) found that the severity of acute GVHD correlated with pulmonary levels of HHV-6 DNA detected by quantitative PCR (110). Propinquity analysis indicated that HHV-6 was the probable cause of three episodes of skin rash and three episodes of skin rash with fever among a group of 26 BMT recipients who were investigated longitudinally (254). HHV-6 DNA was present in the skin biopsy specimen of the one patient with histologically proven GVHD. Finally, another longitudinal investigation of 37 BMT recipients found no correlation between the development of acute GVHD and the detection of HHV-6 DNA in PBMC by PCR (528); there was no mention of attempts to detect HHV-6 DNA in biopsy specimens from patients with GVHD. The finding by several groups of investigators of HHV-6 in relation to GVHD and other skin rashes in BMT recipients is interesting, but much additional work is required before cause-and-effect are established. Of note, the involvement of HCMV in BMT-associated GVHD is controversial (reviewed in reference 65).

Carrigan et al. (79) were the first to report an association of HHV-6 with pneumonitis in BMT recipients. HHV-6 was isolated from the blood and bone marrow of a 19-year-old man who received an autologous transplant for testicular carcinoma and also later died of progressive pneumonitis. IHC staining of lung tissue revealed widespread HHV-6 infection, and no other pathogens were detected. A second case involved a 32-year-old woman treated by allogeneic transplant for myelodysplastic/preleukemia syndrome and who developed interstitial pneumonitis. HHV-6 was cultured from a bronchoalveolar lavage (BAL) specimen, and IHC staining revealed many HHV-6 infected cells. Although HCMV was detected in the same sample by the shell vial technique (192), it was neither isolated in standard cultures nor detected by IHC staining. The patient was treated with two antiviral agents, and her condition slowly improved, with eventual resolution of pneumonitis. She later died of leukopenia and disseminated adenovirus infection. In both of these cases, HHV-6 staining was observed primarily in macrophages. Three other cases of pneumonitis associated with HHV-6 have been described by Carrigan among nine BMT recipients whose lung tissue was studied by IHC staining postmortem (77). Each of the three patients also harbored other potential pathogens, including HCMV, HCMV and adenovirus, and *Aspergillus*, respectively, making analysis of the possible contribution of HHV-6 to pulmonary disease difficult.

Paraffin-embedded lung tissue specimens from seven BMT recipients who died of interstitial pneumonitis were examined by IHC staining with HHV-6-specific MAb (411). Six (86%) of the seven patients had HHV-6 antigens identified in lung tissue, predominantly in pneumocytes (although staining was also observed in lymphocytes and macrophages). However, in none of these cases was HHV-6 the sole potential pathogen identified. Adenovirus antigens were also found in all six patients positive for HHV-6, and staining for HCMV was positive in four (67%) of the six positive for HHV-6. Negligible reactivity with the antibody preparations used was found in lung biopsy tissue from a healthy control. As with the cases cited above, the finding of HHV-6 in combination with other potential pathogens makes it difficult to interpret a possible etiologic association of HHV-6 with pneumonitis in these patients.

Cone et al. studied 15 BMT recipients who developed pneumonitis and found that 6 (40%) had significantly higher levels of HHV-6 DNA in lung biopsy specimens examined by PCR than did the controls (110). The HHV-6 DNA levels were quantified and normalized by comparison to β-globin DNA PCR results. No other pathogen was identified in the specimens from five of these patients. Specimens from the other nine BMT recipients contained HHV-6 DNA levels similar to those observed in specimens from 14 of 15 immunocompetent controls who were HHV-6 seropositive. The severity of acute GVHD in these BMT recipients correlated with the levels of HHV-6 DNA found by PCR. Of additional interest, only one of the six BMT patients with high viral DNA levels died of pneumonitis whereas while six of the other nine died of acute lung disease. This finding suggests that HHV-6-associated pneumonitis may not be as severe as that caused by other infectious agents common in this group of patients. Of note, all BMT recipients and normal controls who were HHV-6 seropositive had positive PCR results, suggesting that HHV-6 is routinely present in lung tissues and that its presence is not necessarily associated with pneumonitis. These conclusions were later extended to both HHV-6A and HHV-6B by the use of variant-specific PCR primers with tissue samples from the same BMT recipients and controls plus additional specimens from surgical patients (110, 114, 115). Nearly two-thirds of lung specimens were PCR positive for both HHV-6 variants (115).

Qualitative PCR was used to look for evidence of HHV-6 infection in buffy coat leukocytes, oral lavage fluid, and urine of 60 BMT recipients (539); 60% of the patients were positive in one or more tests. A correlation was found between HHV-6 PCR positivity and acute GVHD, but there was no other association of disease with positive PCR results. Carrigan (78) has pointed out two difficulties with this study: (i) qualitative PCR does not distinguish latent from productive viral infection, and (ii) although the specimens analyzed are easily obtainable, they have limitations in predicting disease, as exemplified in studies of HCMV (360). The samples evaluated lack the tissue specificity of organ biopsy specimens, and there were no means to detect local immunohistopathologic abnormalities in this experimental design. It is not surprising that only limited correlation with disease was found. Another recent investigation of six BMT recipients examined BAL specimens for DNA of HCMV, EBV, and HHV-6 (422). One patient was PCR positive for HHV-6 alone, and one each was positive for HHV-6 and either HCMV or EBV. Only the patient who was PCR positive for both HHV-6 and HCMV had clinical pneumonitis. As with the preceding study, this investigation had several limitations in technique. Further discussion of HHV-6

and pulmonary disease in immunocompromised hosts, including BMT recipients and persons with AIDS, is presented below.

As described in the section on nomenclature and classification (above), about 90% of the HHV-6 strains detected in BMT recipients have been HHV-6B (146, 147, 178, 254, 458, 528, 539). Carrigan and coworkers have reported several other studies of BMT recipients. In one investigation of 16 BMT patients with idiopathic febrile episodes within the first 100 days posttransplantation, 6 were viremic with HHV-6B (146). Of those positive for HHV-6B, four developed severe bone marrow graft suppression and HHV-6B was cultured from the marrow. HHV-6B has also been isolated from PBMC and bone marrow of other adults with HHV-6-associated disease. In one case, HHV-6B was cultured from PBMC and bone marrow of a liver transplant recipient who developed a febrile syndrome with dermatosis and encephalopathy (467). In another case, an apparently immunocompetent adult who recovered from pneumonitis in which both HHV-6 and *Legionella pneumophila* were obtained from the lungs remained anemic for several months after the onset of illness (198). The nucleotide sequence in each of two variable genomic regions was identical between HHV-6B isolated from a bone marrow aspirate and 2 months earlier from the blood of this patient. Both of these patients are described below in greater detail.

Further investigations have revealed a possible role for HHV-6A in BMT recipient marrow suppression. HHV-6 was recovered from the bone marrow of a BMT patient with graft failure almost 2 years after transplantation (440). HHV-6 was also detected in the marrow biopsy specimen by IHC staining. Both variants were detected in marrow cells cocultured with cord blood mononuclear cells by immunofluorescence with variant-specific MAb; this was confirmed by PCR with variant-specific primers. In separate work, the HHV-6 variant was determined for 18 BMT recipients who were HHV-6 DNA positive by PCR of peripheral leukocytes and/or body fluids. For 15 (83%) of the 18 patients, HHV-6B sequences were found exclusively; for 2 (11%) patients, HHV-6A sequences were found in many specimens obtained over a period of months; and 1 patient was positive for HHV-6A and HHV-6B at different times (539). A third study in which HHV-6A was detected is described below (528). In a fourth study, HHV-6A was detected in plasma by nested PCR from all three (23%) of 13 BMT recipients who were tested for HHV-6 (458).

Another investigation of BMT recipients by Carrigan and Knox involved 15 adult patients who developed posttransplantation bone marrow suppression (81). The patients were divided into two groups: (i) seven with suppression for which a diagnosis had been made, and (ii) eight for whom suppression was idiopathic. HHV-6 was not recovered in cultures of bone marrow aspirates from any of the seven patients with diagnosed bone marrow suppression but was culturable from six of the eight patients with idiopathic cases. Of note, of the six for patients from whom HHV-6 was recovered, five (83%) demonstrated suppression of cells of more than one lineage. This result contrasts to finding multiple cell lineage suppression in only two (22%) of the nine patients with HHV-6-negative bone marrow. HHV-6 was recovered from 4 (18%) of 22 unselected BMT recipients by culture of marrow aspirate 1 month after engraftment; one of these four was later found to have HHV-6-associated bone marrow suppression. This finding compares with the six of eight HHV-6-positive BMT recipients with idiopathic marrow suppression noted above. The differences in HHV-6 recovery and cell lineage suppression between these groups of patients were statistically significant.

In most of the studies described above, HHV-6 activity in BMT recipients was monitored by routinely culturing recipient PBMC for only a short period after the transplant and then only in association with clinical events, such as fever of unknown origin. Kadakia et al. monitored 26 adult BMT recipients (11 autologous and 15 allogenic) for up to 1 year after transplantation by sampling blood and saliva before transplantation and then weekly for 1 month, monthly for 3 months, and between 6 months and 1 year posttransplantation (254). HHV-6 activity was assessed by PCR of patient PBMC and saliva, virus isolation from PBMC, and increases in serologic responses to HHV-6. No virus was isolated by culture from PBMC obtained prior to transplantation, but 12 (46%) of the 26 patients were culture positive at least once after transplantation. Sinusitis was associated with HHV-6 isolation in autologous recipients, and simultaneous HCMV activity was associated with HHV-6 isolation in allogenic recipients. No association was seen with delays in engraftment or marrow suppression. In total, 23 (88%) of the 26 patients were positive for one or more of the HHV-6 markers during the posttransplantation period. Propinquity analysis indicated that HHV-6 activity was associated with 16 clinical events in nine patients, including one death from idiopathic pneumonia. HHV-6 DNA was detected in lung tissue from that patient. An interesting observation was the reduced frequency of detecting HHV-6 by culture during periods of acyclovir treatment. This result is in spite of the low in vitro sensitivity of HHV-6 to the drug.

Wang et al. assayed DNA of various herpesviruses, including HHV-6, HHV-7, EBV, and HCMV, by nested PCR of PBMC from 37 allogenic BMT recipients over a period of up to 1 year following transplantation (528). HHV-6 DNA was detected in specimens from 8 (22%) and 26 (70%) of the 37 patients prior to and at some point after transplantation, respectively. Of the 26 patients with HHV-6-positive samples, 18 (69%) had variant B only. One patient was positive only for HHV-6A, and three were positive for both HHV-6A and HHV-6B. Of the three who were positive for both viruses, HHV-6A was detected only at time points when HHV-6B was simultaneously detected whereas HHV-6B was sometimes detected in the absence of HHV-6A. No specimens were positive for HHV-6A later than 4 weeks after transplantation. The variant could not be determined for the four other patients with HHV-6-positive specimens. Delayed engraftment of granulocytes and of platelets occurred in 5 and 6 of the 37 BMT recipients, respectively. The frequency of HHV-6-positive specimens was significantly higher for patients with delayed engraftment of either kind than for those with normal engraftment. During the first 3 months after transplantation, the frequency of HHV-6-positive specimens was significantly higher for patients with delayed engraftment compared to those with normal engraftment. However, within the same period, there was no correlation between viral DNA-positive samples and engraftments for any of the other herpesviruses. Of note, only three (33%) of nine BMT recipients receiving high-dose prophylactic acyclovir had HHV-6-positive specimens, compared with 23 (82%) of 28 recipients who did not receive this prophylaxis, similar to the results of Kadakia et al. (254). Prophylactic use of high-dose acyclovir has previously been reported to reduce the frequency of HCMV infection in BMT (361, 414) and renal transplant (44) recipients. For both this and the investigations of Kadakia et al., the study methodologies place limitations on the interpretation of the results, as described above.

(ii) Laboratory studies. Initial reports of HHV-6 infection related to bone marrow transplantation indicated that virus could be isolated from the PBMC and bone marrow of many BMT recipients (77, 79, 146, 567). Bone marrow suppression has also been noted in such patients (79, 146), stimulating

interest in the effects of HHV-6 infection on bone marrow cells in vitro. While such work is provocative, caution must be exercised in extrapolating such effects to in vivo bone marrow following transplantation, given the considerably more complex environment in the human host than in the laboratory dish. Nevertheless, interesting insights may be gained from such in vitro investigations.

Both direct cytolysis and virus-induced inhibition have been proposed as mechanisms leading to bone marrow suppression by HHV-6 (73, 146, 279, 555). While direct loss of marrow cells due to viral cytolysis is logical, there is also in vitro evidence for suppression of growth factor-induced colony formation and maturation of bone marrow precursors cells (73, 82, 146, 279). There is little evidence for HHV-6 replication in infected bone marrow cultures (73, 279), but outgrowth of erythroid and granulocyte-monocyte precursors, pluripotent stem cells, and stromal cells are suppressed in virus-infected cultures (146, 279). Both HHV-6A and HHV-6B can suppress such functions in vitro, with recent work indicating greater suppression by the former, as measured by various colony-forming assays, stromal outgrowth, and granulocyte-macrophage colony-stimulating factor response, leading the investigators to conclude that HHV-6A has increased virulence in this respect compared with HHV-6B (82). One study, involving an HHV-6B strain, indicated a possible role for IFN-α in virus-mediated suppression of bone marrow precursors (279). This hypothesis is attractive in light of other work demonstrating induction of IFN-α in HHV-6-infected mononuclear cells in vitro (270). Later work showed that HHV-6A and HHV-6B suppressed the outgrowth of macrophages in granulocyte-monocyte colony-stimulating factor- or IL-3-stimulated cultures, but addition of neutralizing antibodies to IFN-α, IFN-β, or IFN-γ did not reverse such suppression (73). Thus, differences may exist in the mechanisms of marrow suppression for the HHV-6 variants, and other cytokines such as TNF-α or IL-1β, which are inducible by HHV-6A in vitro (165), may be important in mediating marrow-suppressive effects, although this has not been demonstrated.

The studies described above associating HHV-6 with bone marrow suppression, both in vivo and in vitro, are of interest. The work which demonstrates that HHV-6 is not routinely recovered from unselected BMT recipient marrow but is often found at this site in BMT recipients with idiopathic marrow suppression is particularly provocative and strongly suggests that HHV-6 is an important pathogen of the bone marrow in this patient population. Although care must be taken in its interpretation, in vitro work to date is supportive of this concept. An etiologic role for HHV-6 of either or both variants has not been proven in BMT graft suppression, and in one study no association was seen between HHV-6 activity and graft suppression (254). However, the accumulating in vivo and in vitro data are consistent with the possibility that HHV-6 activity contributes to graft suppression under some circumstances.

Other transplants. Little information is available regarding HHV-6 infection of orthotopic liver transplant recipients. One report described primary infection, documented by both seroconversion to HHV-6 and viremia during a febrile episode, following transplant of a liver from a seropositive donor (530). A syndrome consisting of fever, rash, thrombocytopenia, and encephalopathy associated with HHV-6 viremia was seen in an orthotopic liver transplant recipient and a bone marrow biopsy specimen that was HHV-6-positive by IHC (467). In a study of 50 orthotopic liver transplant recipients, 7 patients seroconverted or had a significant rise in titer to HHV-6 but had no serologic evidence of active HCMV infection (493). Fever and neurologic abnormalities were more commonly observed in this group than in a group of 12 patients whose HHV-6 status was unchanged, although the numbers in each group were small.

HHV-6 serologic findings were studied in a group of 58 heart transplant patients (433). Five patients who developed posttransplantation hepatitis and three who did not had HHV-6 seroconversion or significant antibody titer increase. HHV-6-specific IgM, but not HCMV-specific IgM, was observed in six patients. The authors concluded that HHV-6-associated hepatitis is not common following cardiac transplantation. No in situ analysis was done to document the presence of HHV-6 in hepatic tissue, and the possibility of cross-reacting antibodies to HHV-7 was not ruled out. The role of hepatic HHV-6 infection in this and other transplant populations requires further study.

Other hosts without HIV. As recounted in a recent publication, a 32-year-old man treated with multiple courses of chemotherapy for ALL experienced an exanthem involving his shoulders, neck, face, and trunk during periods of leukopenia (182). Resolution occurred with recovery of the bone marrow. A skin biopsy specimen of an active lesion demonstrated nonspecific dermatitis with infiltrating lymphocytes. HHV-6 DNA, but not that of HSV, VZV, or HCMV, was detectable by PCR of nucleic acids extracted from the lesion. IHC staining of the lesion was positive with an HHV-6B-specific MAb. The HHV-6 IgG level in serum rose slightly during the exanthem, without detectable anti-HHV-6 IgM. After several recurrences, treatment with ganciclovir resulted in disappearance of the exanthem prior to bone marrow recovery, and the lesions did not return. The clinical course and laboratory results are consistent with this being a recurrence of active HHV-6 infection in a host with chemotherapy-associated immunosuppression. Lack of significant humoral response to HHV-6 may have been due to underlying drug-induced immunosuppression.

Two case reports have described patients with serious HHV-6-associated illness, including pneumonitis, who were HIV-1 seronegative and were not receiving pharmacologic immunosuppression when disease began. The first case involved an infant with an immunodeficiency of unknown etiology, associated with progressive T-cell lymphocytopenia, who eventually died at age 13 months with high levels of HHV-6 present in the lymph nodes, thymus, and lungs (415). HHV-6A was the predominant variant identified in these tissues. HHV-6-infected cell density in the lungs was similar to that in the young BMT recipient described previously (79, 81). Shortly before death, *Pneumocystis carinii* and HCMV were detected in BAL specimens, prompting treatment with multiple agents. At autopsy, no evidence of *P. carinii* or HCMV was found. HHV-6-specific IgM and decreased $CD4^+$ and $CD8^+$ cell counts were documented as early as 4 months of age, although the time of primary HHV-6 infection was not known. The authors of the report hypothesized that this child's immunosuppression was due to HHV-6A infection which eventually resulted in fatal HHV-6 pneumonitis and that the other microorganisms contributed to the pathogenesis.

In the second case, an apparently immunocompetent 37-year-old man developed pneumonitis with *Legionella pneumophila*, documented by culture from a bronchoscopy specimen, positive urine antigen, and elevated serologic titers (442). Early in the course of his illness, the patient's total leukocyte count was elevated but he was lymphocytopenic. Despite extended treatment with appropriate antibiotics, he progressed to multiorgan failure, requiring mechanical ventilation and hemodialysis. High-dose methylprednisolone therapy was instituted, and the patient's condition rapidly improved. HHV-6 was cultured from peripheral blood several weeks after disease

onset and was later identified as variant B. HHV-6 antigen, revealed by IHC staining with a rabbit anti-HHV-6 polyclonal antiserum, was detected in a lung biopsy specimen. Staining was detected in a moderate number of cells, mostly macrophages, distributed throughout the tissue. The patient's anti-HHV-6 IgG titer rose from 500 near the time of disease onset to 32,000 at discharge, and his anti-HHV-6 IgM titer was positive for his entire hospitalization, eventually becoming negative during rehabilitation. The patient's age and HHV-6 serologic results suggest viral reactivation, but reinfection was not ruled out. Dexamethasone inhibited in vitro replication of the strain of HHV-6 recovered from the patient's blood. The investigators suggested that the beneficial effects of corticosteroid therapy observed in vivo were due to a local anti-inflammatory effect and suppression of HHV-6 replication. Whether there was a direct antiviral effect due to corticosteroid treatment in this case is unclear. Although this patient was outwardly immunocompetent when his illness began, local pulmonary defense mechanisms may have been diminished due to his infection with *L. pneumophila*, allowing HHV-6 to act as an opportunistic pathogen during reactivation.

These two cases illustrate the potential for idiopathic immunosuppression or pulmonary infection with another microorganism to contribute to susceptibility to HHV-6-associated pneumonitis. Further discussion of HHV-6 and pulmonary disease is presented below.

AIDS. Considerable attention has been focused on possible associations of HHV-6 and the pathogenesis of AIDS. This stems from the fact that both HHV-6 variants are members of the herpesvirus family and that all the previously described human herpesviruses cause disease in immunocompromised hosts. In addition, HHV-6 replicates in $CD4^+$ T cells, the primary HIV-1 target. Studies of molecular interactions between HHV-6 and HIV-1 in vitro are described above. Here we discuss what is known with regard to HHV-6-associated disease in HIV-1 seropositive patients.

Studies of serologic responses to HHV-6 in HIV-infected persons have yielded widely varying results. Comparisons between HIV-infected persons and uninfected controls have shown either no difference in seroprevalence and titer (67, 159, 173) or higher (4) or lower (93, 472) prevalence among those infected with HIV. One study found no correlation of HHV-6 seroprevalence with progression of AIDS (472), while another found slow progressors had decreased HHV-6 seroprevalence compared with those whose disease advanced rapidly (93). Peripheral $CD4^+$ counts increased slightly in HIV-1-infected children with both anti-HHV-6 IgG and IgM but declined significantly in those with anti-HHV-6 IgG alone (384). Other indicators of HIV-1-related disease showed no or inverse correlation with the presence of HHV-6 IgM. It is obviously difficult to interpret such disparate results. We assume that some of the differences are due to differences in assay conditions. Given that HHV-6 infects $CD4^+$ T cells, which are important in the generation of a humoral immune response, the differences between case and control populations, regardless of the direction of the measured change, may reflect the ability of the host to mount a humoral response as much as it does true pathologic effects of HHV-6.

Several investigations have used PCR to examine the prevalence and in some cases the quantity of HHV-6 DNA in saliva and blood of HIV-seropositive hosts (136, 140, 160, 189, 201). The results of such studies have shown great variation, which is not unexpected, given the differences in the patient populations examined, sample preparation, and PCR methodology. The frequency of HHV-6 DNA detection in saliva has ranged from 3 to 63% for those uninfected with HIV (136, 160, 189, 201) and from 0 to 96% for HIV-infected individuals (136, 160, 189, 201). Neither the frequency of HHV-6 detection nor the quantity of HHV-6 genomes amplified was related to the peripheral $CD4^+$ count in two studies (160, 189). In one of these studies, the amount of HHV-6 DNA in saliva also did not vary between HIV-1-seropositive patients and healthy controls studied previously by the same laboratory (112, 160).

Reported frequencies of detecting HHV-6 DNA in PBMC have been highly variable, as described for saliva. HHV-6 DNA was detected in 10 to 49% of those uninfected with HIV, while 10 to 75% of HIV-infected individuals had HHV-6 DNA identified (160, 189, 201). In one study, HHV-6 DNA was detected at a higher frequency in HIV-seronegative persons than in HIV-seropositive individuals (201), while in another study there was no difference (189). The frequency of detecting HHV-6 DNA in PBMC decreases as $CD4^+$ counts decrease (140, 160, 189, 201). This correlation is possibly due to loss of $CD4^+$ cells from the PBMC pool. The one study examining HHV-6 variant distribution in saliva or PBMC from HIV-seropositive patients found variant B exclusively in 64 specimens, with variant A identified in 1 (4.8%) of 21 samples from HIV-seronegative individuals (189).

These investigations of HHV-6 in the saliva and PBMC of persons infected with HIV have not indicated a synergistic relationship between HHV-6 and HIV in the pathogenesis of AIDS. As noted above, PCR studies with saliva or blood samples from normal subjects have also shown widely varying results, for a variety of possible reasons. Solid lymphoid tissue may be a more useful substrate for studies of possible in vivo interactions between HIV-1 and HHV-6. Studies with lymph nodes from HIV-infected persons have yielded rates of PCR positivity for HHV-6 DNA or detection of HHV-6 antigens by IHC staining in excess of 50%, and in some reports the percentage was much higher (105, 116, 140, 141, 164, 281, 284, 457, 517). Variant A was detected more frequently than variant B in two studies (164, 284) but not in a third (140).

Recent work by retrovirologists has shown that lymph nodes are heavily infected with HIV during the clinically latent period of infection and that elevated levels of HIV replication are instrumental in disease progression (107, 155, 220, 397, 398, 534). The histopathologic pattern of follicular hyperplasia occurs during this period. The term "follicular hyperplasia," as used in this review, refers to a reactive or benign hyperplasia (394) that is distinct from the angiofollicular hyperplasia of Castleman's disease. One study demonstrated that during clinical latency the frequency of virus-infected cells and rates of viral replication were strikingly increased in lymph nodes compared to PBMC from the same person (397). Separate work involving in situ PCR showed that up to 25% of $CD4^+$ T lymphocytes present in lymph node germinal centers are infected with HIV (155). As disease progresses, follicular hyperplasia shifts to follicular involution, with nearly complete loss of lymph node structure and function (396). These investigations suggest that lymphoid tissue, including lymph nodes obtained during the clinically latent period of HIV infection, may be the best clinical specimens for studying a potential role of HHV-6 in the development and progression of AIDS.

Early HIV-1 seroconverters were found to have occasional cells which contained HHV-6 DNA detectable by ISH in peripheral lymph nodes and spleen (<1 infected cell per 10^5 nucleated cells) (347). The frequency and distribution were similar to those found in lymphoid tissues of other groups undergoing biopsy for various lymphoproliferative conditions (294). The low frequencies observed were interpreted as characteristic of latent infection (294, 347). In another investigation (140), HHV-6 DNA was detected by PCR (albeit at low levels)

in lymph nodes of 13 (65%) of 20 HIV-seropositive patients with nonneoplastic reactive lymphadenopathy but in similar lymph nodes of only 2 (20%) of 10 HIV-seronegative patients. In 12 (92%) of the 13 specimens from HIV-seropositive patients, HHV-6B was identified; in the remaining specimens, both variants were detected. All 13 HHV-6-positive specimens from HIV-seropositive hosts demonstrated follicular hyperplasia. Four HHV-6-negative specimens from HIV-seropositive patients showed follicular involution. HIV-1 p24 was detected in germinal centers of 19 (95%) of the 20 lymph nodes from HIV-seropositive patients.

Active HHV-6 infection in lymph nodes was demonstrated in HIV-seropositive patients whose only clinical manifestation was lymphadenopathy (284). IHC staining of 10 lymph node specimens with histopathologic evidence of either follicular hyperplasia or follicular involution with histiocytosis and reactive lymphadenitis revealed the presence of HHV-6 antigens within infected cells in all cases. The use of variant-specific MAb indicated that HHV-6A-infected cells were located in all regions of the lymph nodes, frequently in foci within germinal centers. HHV-6B-infected cells were found in areas with high T-lymphocyte concentrations, such as medullary and parafollicular domains. HHV-6A was detected in the majority of infected cells, with HHV-6A/HHV-6B ratios ranging from 2:1 to 20:1 in various specimens. In six of eight patients, absolute $CD4^+$ lymphocyte counts were above 200/mm^3 when biopsy specimens were obtained. Normal lymph nodes or those with follicular hyperplasia obtained from HIV-seronegative persons were all HHV-6 negative by IHC staining. The apparent ease of detecting HHV-6 antigens in this study contrasts with the low levels of HHV-6 DNA detected in the study of reactive lymphadenopathy in HIV-seropositive patients described above; this apparent paradox may reflect the bulk tissue sampling used in the PCR study compared with the use of IHC to examine defined regions of interest. These studies suggest a pathogenic role for HHV-6 in the involution of lymphoid tissues as HIV infection progresses to a more advanced stage (140, 284).

Recent studies involving quantitative PCR methods have indicated that there may be approximately 0.5 to 5 HHV-6 genomes per 100 cells in lymphoid tissues of AIDS patients (105, 457). In conjunction with previous studies of HIV infection of lymphoid tissue (155, 397), this suggests that there is opportunity for dual infection of $CD4^+$ T lymphocytes, and possibly macrophages, with both HIV and HHV-6. Investigations of such a possibility should be conducted, with attention given to which HHV-6 variant might be involved. The potential for interactions is not limited to simultaneously infected cells, however, because alterations in the production of cytokines or other immune system modulators by cells singly infected with either virus could influence the course of infection in cells that harbor the other virus and are present in the same lymphoid microenvironment. Lymphoid tissue specimens appear to be particularly attractive for future studies of the potential for interactions between HHV-6 and HIV-1 in vivo.

AIDS patients with late disease have widespread dissemination of HHV-6 into many organs. One PCR-based study of five patients who died of AIDS found that HHV-6 DNA was present in 85% of specimens obtained from diverse tissues whereas only 54% of similar tissues were positive in two HIV-negative controls (116). Another investigation involved IHC staining of lung, lymph nodes, spleen, liver, and kidney specimens from nine persons who succumbed to AIDS and found a major HHV-6 antigen in all 34 samples examined (281). Of the 34 specimens, 9 (26%) were also positive for HCMV antigens. Macrophages were the predominant HHV-6-infected cell type in the lungs, spleen, and lymph nodes of most of the patients. The last two tissue types were lymphocyte depleted in most cases, but a lymph node from one patient contained many HHV-6-infected lymphocytes. Counting the number of HHV-6-infected cells per square centimeter of section allowed an approximation of the relative infected-cell burden in the various tissues. The estimates ranged from 4 to 190 infected cells/cm^2 in the lungs for eight of the nine patients, and the ninth patient had 1,578 infected cells/cm^2, similar to the density observed in lung tissue from the 19-year-old BMT recipient described above who died of an HHV-6-associated pneumonitis (79). No HCMV-infected cells were detected in the lung sample from this AIDS patient. The HHV-6 distribution in 11 different tissues obtained at autopsy from seven AIDS patients and three controls was studied by quantitative PCR (105). Approximately equal percentages (67 to 72%) of specimens were positive in both patients and controls, but the median load was higher for AIDS patients than for controls (56 and 10 copies/μg, respectively), with the only statistically significant tissue-specific difference being in the lungs.

HHV-6 DNA, RNA, and proteins have been detected in areas of AIDS-associated retinitis, often in conjunction with HIV-1 or HCMV DNA or antigens (418, 419, 421, 431). Gene products of both HIV-1 and HHV-6 were detected in over half of 17 normal-appearing retinas from persons with AIDS (418). Five other retinas with microlesions of uncertain etiology showed antigens and transcripts of both HIV-1 and HHV-6 but not HCMV; it is unclear whether individual cells were dually infected. These data suggest that local infection with HIV-1 and HHV-6, alone or in combination, may predispose to the development of AIDS-associated retinitis. The significance of the presence of HHV-6 DNA in corneal tissues of some HIV-1-seropositive hosts is uncertain (420).

Possible associations of HHV-6 and CNS disease in AIDS patients are described above. HHV-6 and AIDS-related neoplasia is discussed below.

The possibility that specific organ disease is caused by HHV-6 infection in AIDS patients has been a point of controversy in the recent medical literature. Much of the controversy has focused on whether HHV-6 causes pneumonitis in persons with AIDS (as well as in BMT recipients). In a retrospective study of 36 patients with pneumonia, including 15 with HIV infection, only 1 (7%) BAL specimen from the 15 HIV-seropositive patients was PCR positive, compared with 4 (19%) from the other 14 patients (114). The investigators noted that this finding may indicate that HIV-infected patients are no more prone to pulmonary infection with HHV-6 than are other patients with pneumonitis. However, the immunohistopathologic findings within the infected tissues were not studied, a limitation noted previously in other studies of BMT recipients.

A second investigation was performed by PCR for HCMV and HHV-6 DNA to examine BAL fluid from HIV-1-seropositive individuals with symptoms suggestive of respiratory infection, including cough, dyspnea, and fever (413). $CD4^+$ T lymphocytes comprised 4 to 15% of peripheral lymphocytes, suggesting that some patients were at significant risk of opportunistic disease. Of 34 patients tested, 12 (35%) were HCMV positive by PCR, and 10 of these (30% of total) also had HCMV isolated from BAL fluid in tissue culture. Confirmation of PCR results was obtained by nested PCR in two cases which were culture negative. In contrast, none of the BAL fluids demonstrated the presence of HHV-6 by either nested PCR or viral culture. The HHV-6 primers and procedures had been used successfully in PCR assays by others (35, 131). Several limitations should be noted with this study. First, lower respiratory tract disease was not documented for many of these

patients. Whether there was sufficient clinical information to conclude that pneumonitis was present is unclear. Second, apparently none of the patients underwent biopsy to demonstrate potential pathogens within lung tissue.

Lung tissue specimens are generally considered to be the most reliable source of diagnostic material for establishing HCMV as the cause of pneumonitis in immunocompromised patients (85, 145, 399, 471). Several groups have found that the detection of HCMV in BAL fluids does not correlate with the presence of or subsequent development of HCMV pneumonitis (158, 479). Thus detection of HCMV DNA by PCR with BAL fluids from HIV-seropositive patients may be of little value in evaluating the possible causation of pneumonitis by this virus. This may also be the case for HHV-6. In spite of the limitations of the studies of HHV-6 in BAL fluids of HIV-seropositive hosts, the results suggest that HHV-6 is not often associated with pulmonary disease in these patients.

Several views have been expressed of the potential for HHV-6 to cause pneumonitis in immunocompromised hosts. Some of these views incorporate hypotheses concerning possible pathogenic mechanisms for HCMV-related pneumonitis in BMT recipients. For HCMV, it has been proposed that interstitial pneumonitis may be due to direct effects of viral replication in the lungs, to immunopathologic effects initiated in response to HCMV infection, or to a combination of these mechanisms (reviewed in reference 65). According to Griffiths et al. (205), HHV-6 is not necessarily an etiologic agent of pneumonitis (or of bone marrow suppression) in immunodeficient patients. They noted that Koch's postulates have not been fulfilled and suggested that the HHV-6 load in the lungs has not been shown to correlate with pneumonitis. Rather, they argued that studies in BMT and AIDS patients are consistent with HCMV pneumonitis being an immunopathologic disease that does not correlate with HCMV levels. In their view, investigations that have quantified HHV-6 levels in lungs in relation to disease should be viewed similarly, there not necessarily being a correlation. In contrast, Knox and Carrigan (282) observed that in their studies, active HCMV infection of the lungs was universally accompanied by HHV-6 infection, which was not addressed in previous work. They also have emphasized that BMT patients with profound lymphopenia have high rates of HCMV pneumonitis (110) whereas those with HCMV-specific cytotoxic T-lymphocyte responses are more strongly protected from such lung disease (430). This is the opposite of what would be predicted by the immunopathology theory. Knox and Carrigan instead favor the hypotheses that both HCMV- and HHV-6-associated lung diseases correlate directly with the quantity of productively infected cells present.

Cone et al. have also discussed possible pathogenic mechanisms which may be involved in putative HHV-6 pulmonary disease (114). They observed that direct damage to lung epithelial cells is possible, given that various studies have demonstrated HHV-6 infection of some epithelial cell types, possibly including human pneumocytes (411). They note, however, that lysis of such cells has not been demonstrated. Other possible mechanisms include interactions with other pulmonary pathogens, such as HCMV or adenovirus. HHV-6 and HIV-1 coinfection of macrophages, which has been shown to kill 70% of the cells compared with 2% for HIV infection alone in vitro (280), could deplete the lungs of a cell type essential for pulmonary immunity. HHV-6 induction of cytokines, including IFN-α (270, 496), IL-1, and TNF-α (165, 202), has also been proposed as potentially enhancing HHV-6-induced pulmonary disease (114).

Cone et al. (114) cautioned against ready acceptance of HHV-6 as a pulmonary pathogen because of (i) the limited number of well-documented cases, (ii) the ubiquitous nature of HHV-6 infection, (iii) the lack of a well-defined clinical presentation, and (iv) the absence of a clearly defined pathogenetic mechanism. They also observed that there may be a bias in case selection due to acquisition of pulmonary specimens mostly from severely ill patients and from autopsy, which could lead to an apparent but insignificant association of HHV-6 with severe pneumonitis.

Lusso and Gallo (337) describe the most important issue with respect to HHV-6-related disease in HIV-positive persons as being not so much a direct effect on specific target organs as the putative cofactor role of HHV-6 in contributing to immune deficiency of the host. We agree with the suggestions of these authors and of Cone et al. that clarification of the role of HHV-6 in diseases of persons with HIV infection will require (i) longitudinal studies of HIV-infected individuals by quantitative molecular techniques, (ii) coinfection studies with experimental animal models, and (iii) well-controlled prospective clinical trials of therapeutic agents with demonstrated efficacy against the putative cofactor.

Neoplasia

Molecular mechanisms. As for several other herpesviruses, there has been interest in exploring the potential for HHV-6 to act as an oncogenic agent. HHV-6 genomic DNA and several cloned fragments can transform NIH 3T3 cells and human keratinocytes in vitro to a form that is tumorigenic in nude mice (426, 427, 507). The cloned fragments with transforming activity map to the DR and to the betaherpesvirus gene block (Fig. 2). These regions coincide with regions known to encode genes that can transactivate the HIV-1 LTR. It will be interesting to determine whether the transactivating and transforming activities are encoded by the same ORF.

HHV-6A can infect human cervical carcinoma cell lines immortalized with human papillomavirus type 16 (HPV-16) and HPV-18, although no cytopathic effect or productive infection was observed (94). The HHV-6 DNA was present as an episome, with expression of early-late viral proteins; expression of HPV RNAs encoding the oncoproteins E6 and E7 was enhanced. These HHV-6-infected, HPV-transformed cell lines formed tumors in nude mice more rapidly than did non-HHV-6-infected control lines.

In contrast to the ability of some HHV-6 genomic segments to transform cells, rep_{H6A}, the HHV-6A homolog of rep_{AAV-2} (described in the section on transcription, above) (509), can suppress H-*ras* transformation of NIH 3T3 cells (24). H-*ras* is activated in numerous human malignancies (15, 126, 183, 195, 290, 291, 452, 461, 571). Tumor suppression in the transformation experiments was via down-regulation of H-*ras* transcription rather than by activation of the H-*ras* p21 protein. In addition, rep_{H6} can suppress HIV-1 LTR transcription (24).

Clinical associations: lymphoproliferative disorders. Clinical investigations of a possible relationship between HHV-6 and neoplastic disease have focused primarily on lymphoproliferative disorders. This is not surprising, given the lymphotropism of both HHV-6A and HHV-6B. Comparison of results obtained in various studies is complicated by differences in detection methods, in sensitivities of specific methods, in patient populations, and in the percentage of tumor cells with respect to the total cellular mass examined. In situ localization methods are of particular value in determining the cell type that harbors the virus, but they are limited by a lack of sensitivity relative to PCR and have been used infrequently. We will deal with individual kinds of malignancies separately.

(i) Non-Hodgkin's lymphomas. HHV-6 DNA was detected by blot hybridization in 5 of 157 specimens of tissues of patients with non-Hodgkin's lymphoma (NHL) (246, 251). Two of these were B-cell lymphomas in the context of Sjögren's syndrome. HHV-6 DNA was also found by nucleic acid hybridization in 1 of 14 lymphoma tissue specimens obtained from patients with Sjögren's syndrome (176). There were no positive results among 20 salivary gland tissue samples from patients with Sjögren's syndrome without lymphoma.

HHV-6 DNA was detected by PCR in biopsy specimens from 45 (59%) of 76 patients with NHL (18 positive results from 29 lymphomas of B-cell origin) (492). Two other groups of investigators found just 3 NHL specimens positive for HHV-6 DNA by PCR among 113 examined (140, 514). At least one of the positive specimens was HHV-6 negative by blot hybridization (140). These 113 NHL cases appear to be expansions of previously reported series (133, 513). One of the groups concluded that HHV-6 is unlikely to play a major etiologic role in the development of B-cell NHL (133). Several studies have used ISH to search for evidence of HHV-6 in tissues from NHL patients. In one investigation, 2 of 16 samples from NHL patients were positive for HHV-6 DNA by PCR and ISH (60). Blastic B cells appeared to be involved in both cases. Of 45 specimens from NHL patients, 8 (18%) were positive for HHV-6 by ISH in a separate study (560). Viral DNA was detected in the nuclei of cytologically defined tumor cells in a majority of positive specimens. In both of these studies, HHV-6 DNA was detected in small clusters or scattered within the tissue. An additional investigation involving ISH observed HHV-6-positive cells in at least seven tumor specimens from NHL patients at a frequency similar to that seen in reactive lymphadenopathy (<1 cell per 10^5 nucleated cells) (294). Positive cells were small and lymphoid. In one sinusoidal B-cell lymphoma, the frequency was slightly increased over that of the control tissue.

Evidence for possible HHV-6 involvement in NHLs of the CNS was sought in a recent investigation of paraffin-embedded tissue from 37 patients with sporadic primary cerebral lymphoma (PCL) and autopsy tissue from 3 AIDS patients with PCL (404). Most of the sporadic PCLs and all the AIDS-related PCLs were high-grade B-cell lymphomas. Only 1 (3%) of the 39 sporadic PCLs and none of the AIDS-related PCLs were positive for HHV-6 DNA by PCR and hybridization blotting. In contrast, all three AIDS-related PCLs but none of the sporadic tumors were positive by dot blotting for EBV DNA. Previous work has shown the presence of the EBV genome in 50 to 100% of PCLs of AIDS patients (346, 370). These investigators concluded that HHV-6 is unlikely to play a significant role in the pathogenesis of PCLs. The results of a study exploring a possible HHV-6 association with neuroglial brain tumors were presented above.

Specimens from a small series of patients with angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) and AILD-like lymphomas (AILD-L) were examined for HHV-6 DNA by PCR (333). HHV-6 DNA was found in four of eight and three of four patients, respectively, with AILD and AILD-L. In the five cases analyzed, HHV-6A was found in one patient each with AILD and AILD-L, HHV-6B was found in two patients with AILD-L, and both variants were detected in the fifth patient. EBV DNA was detected in three of seven patients examined. No type of investigation other than PCR was done.

In the series of NHLs described above, only 20 of the tumors were reported to have come from HIV-seropositive hosts (140, 404); the HIV status was not reported for over 300 other patients, including those for whom HHV-6 DNA was detected by ISH. Most patients in these studies did not appear to be at high risk for HIV infection. In a study by Dolcetti et al. (140), 17 HIV-related and 35 HIV-unrelated NHLs were directly compared; there was no significant difference in rates of detection of HHV-6 DNA by PCR (6 and 0%, respectively). However, EBV DNA was detected by PCR significantly more often in HIV-related (53%) than in HIV-unrelated (32%) tumor specimens. This group included all 64 patients with of NHL or related disorders for whom specimens were examined for the presence of HHV-6 DNA by ISH in two investigations (60, 560) and at least 5 of 7 similarly studied patients in another series (294).

Two recent reports (164, 517) described investigations of tumor tissues for HHV-6 DNA by PCR in which a majority of specimens studied were obtained from HIV-seropositive hosts. Fillet et al. found that 12 (44%) of 27 tumor specimens obtained from various tissues of patients with AIDS-associated NHL were positive for HHV-6 DNA (164). Of 15 lymph node specimens, 8 (53%) were positive. For comparison, 7 (35%) of 20 tissue specimens obtained from HIV-seronegative patients with NHL were also positive, including 5 (36%) of 14 lymph node specimens. HHV-6 DNA was also found in two (50%) of four and five (55%) of nine lymph node specimens from HIV-seropositive and HIV-seronegative persons, respectively, with follicular hyperplasia. The prevalence of HHV-6 DNA was similar in tissue specimens from patients with AIDS-associated NHLs when compared to tissue specimens from other control groups individually as well as together. The frequency of detection of HHV-6 DNA was similar in lymph node specimens (48%) and other tissues (36%) for all samples. Interestingly, for AIDS-associated NHLs, both HHV-6 variants were found in six patients, only variant A was found in one patient, and only variant B was found in two patients. The variants were similarly distributed in the other groups of patients. Estimates of detectable HHV-6 DNA copies per cell were low, less than one copy per 750 cells in most samples.

The study by Fillet et al. (164) contrasts with the work of Di Luca and coworkers (133, 140) in several respects. First, Fillet et al. observed a higher rate of detection of HHV-6 DNA in tumor and lymph node specimens from both HIV-positive and HIV-negative NHL patients than did Di Luca and colleagues, even though the frequencies were similar between the groups in the two studies. Second, Fillet and colleagues found similar rates of HHV-6 DNA detection in nonneoplastic lymph nodes from AIDS patients and HIV-seronegative patients, while Di Luca et al. found a significantly higher frequency in specimens from patients with HIV-associated lymphadenopathy than from HIV-seronegative patients. These differences may have resulted from differences in the patient populations examined (including the stage of HIV-associated disease), sample preparation and handling, and PCR methodology. Also, the numbers of specimens examined from many of the groups of patients were quite small. Neither study, however, demonstrated significant differences in HHV-6 DNA detection between patients with HIV-related or HIV-unrelated NHLs.

In separate work by Trovato et al. (517), tissue specimens from patients with AIDS-related lymphoproliferative disorders were examined by PCR. HHV-6 DNA was detected in 16 (89%) of 18 B-cell specimens from NHL patients and in 3 (100%) specimens from Hodgkin's disease (HD) patients. Only HHV-6A DNA was identified in the two NHL patients in which the variant was determined. HHV-6 DNA was also found in non-lymphatic tissues in nine (64%) of 14 specimens from the same patients. The high proportion of HHV-6-positive tissue samples, including those from patients with and without lymphoma, may reflect the wide dissemination of

HHV-6 infection in persons with late-stage AIDS (116, 281) rather than a specific association with their malignancies.

NHLs are frequently clonal. Thus, the information described above may be interpreted to indicate the lack of an etiologic association between HHV-6 and the malignant process in most, if not all, of the NHLs in which HHV-6 DNA was detected. These include (i) the small proportion of virus-infected cells in HHV-6-positive NHL specimens from (presumably) HIV-seronegative hosts; (ii) the low copy number in HHV-6-positive, AIDS-associated NHLs; (iii) the similar rate of detection of HHV-6 DNA in HIV-related NHLs and HIV-unrelated NHLs in two studies and nonneoplastic lymphoid tissues from control patients in one of the two investigations. The frequencies of HHV-6 detection in both types of NHLs in the other study were very low. One possibility not ruled out by the data is that a "hit-and-run" mechanism involving HHV-6 might be operating. Nevertheless, there is no clear proof of an etiologic association between HHV-6 and any type of NHL.

(ii) Hodgkin's disease. Of perhaps greater clinical interest is work involving HHV-6 in HD patients. One study reported that lymphocyte specimens from 3 (12%) of 25 patients with HD were PCR positive for HHV-6 sequences (513). All three cases were of the nodular sclerosis-lymphocyte depletion subtype, and the specimens from two of these were also positive by less sensitive Southern blot DNA hybridization. It should be pointed out that many studies of the nodular sclerosis type of HD, which accounts for 40 to 60% of HD cases, do not specify a nodular sclerosis subtype. The clinical significance of division into subtypes is not clear (501). EBV DNA was also detected by PCR in the three HHV-6-positive specimens, but with a markedly lower signal intensity than that of HHV-6 (513). It appears that an additional 14 cases of HD which were HHV-6 negative by PCR were added to this series in a later publication (514). Di Luca et al. found that 13 (29%) of 45 tissue samples from HD patients were positive by nested PCR for HHV-6 DNA, although no positive specimens were detected by blot hybridization (133) (Table 4). Most PCR-positive cases involved nodular sclerosis HD, and in all but one case, HHV-6B was the variant detected. Di Luca et al. noted that Reed-Sternberg (RS) cells, which appear to be the neoplastic cellular element in HD, constitute only a small proportion of cells in HD tissue and are often widely scattered in the specimens (133). Thus, the possibility that the relatively rare RS cells harbor HHV-6 DNA detectable by PCR but not by blot hybridization in some cases may be of significance in HD. The location of HHV-6 DNA within the tumors was not determined in the studies by Torelli et al. or Di Luca et al. Of the 13 HHV-6-positive specimens in the latter study, 6 were also positive for DNA of EBV, another virus postulated to have an association with some cases of mixed-cellularity type HD (66, 124, 206, 212, 216, 375, 395, 518, 544, 572).

In separate work, ISH was used to examine tumor specimens from at least seven patients with HD, three of whom had the nodular sclerosis type (294). Most specimens had an increased frequency of HHV-6-positive cells, compared with tissue samples from patients with other lymphoproliferative disorders including various NHLs and reactive hyperplasia. The presence of HHV-6 nucleic acids in RS cells was not specifically addressed, although HHV-6-positive cells were apparently small and lymphoid in appearance.

Another recent investigation did find RS or HD cells expressing HHV-6 nonstructural and/or structural antigens that were detected by IHC staining with MAb in up to 20% of the 26 patients with HD studied (293). HD cells usually accompany RS cells; they are nondiagnostic and are thought to be RS precursors (203). Of the biopsy specimens from HD patients, 77% were positive for HHV-6 DNA by PCR. Other findings of this study included expression of IL-6 receptors, as well as expression of the proto-oncogenes *fes* and *met*, in various cell types. These investigators concluded that HHV-6 may contribute to the development of HD by stimulation of cell proliferation and deregulation of cytokine control of cell function and proliferation rather than by direct oncogenic involvement. Some of the interpretations in the report regarding the expression of viral genes and their relationship to HHV-6 replication and latency are open to question.

TABLE 4. HHV-6 and EBV in lymphomas[a]

Sample[b]	No. of samples positive/total no. (%) for:			
	HHV-6	HHV-6A	HHV-6B	EBV
Healthy donor PBL	8/45 (17)	2/45 (4)	6/45 (13)	ND[c]
NHL				
HIV negative	0/35			ND
HIV positive	1/10 (10)		1/10 (10)	ND
HD				
HIV negative	13/45 (29)	1/45 (2)	12/45 (27)	17/45 (38)
NS	9/30 (30)		9/30 (30)	8/30 (27)
MC	3/10 (30)	1/10 (10)	2/10 (20)	7/10 (70)
LP	1/4 (25)		1/4 (25)	1/4 (25)
LD	0/1 (0)			1/1 (100)

[a] Reprinted from reference 133 with permission of the publisher.
[b] PBL, peripheral blood lymphocytes. HD histotypes: NS, nodular sclerosis; MC, mixed cellularity; LP, lymphocyte prevalence; LD, lymphocyte depletion. Two MC, three NS, and one LP specimens were positive for both HHV-6 and EBV.
[c] ND, not done.

A small study employing a quantitative PCR method (457) found a variable number of HHV-6 genome equivalents per microgram of lymphoid tissue DNA among five patients with HD (type not specified) and five controls (as well as among five patients with AIDS). The number of HHV-6 genome equivalents ranged from less than 10 per μg of total DNA to between 100 and 1,000 per μg for both HD patients and controls (with one control sample in excess of 1,000 genome equivalents per μg). The small number of specimens analyzed, the preselection of tissues for HHV-6 PCR positivity, and the lack of description of HD type limit the ability to make comparisons between the groups tested.

In contrast to the work by Di Luca et al. (133) described above, a recent investigation (516) of nodular sclerosis-type HD in Italian children younger than 15 years old found only 1 (7%) of 15 lymph node specimens positive for HHV-6 DNA by PCR while 5 (33%) were similarly positive for EBV DNA, which was not detectable by ISH. The single HHV-6-positive specimen also contained EBV DNA. In two other ISH studies of HD patients (19, 90), EBV nucleic acids were detected in RS cells of 94 to 100% tissue specimens from Latin American children but only in 36% of those from U.S. children (19). The combined results of these and many other investigations of the frequency of association of EBV and HD have suggested that variations in frequency are linked to geography and socioeconomic criteria, age, ethnicity, immunosuppression, and histologic subtypes (19, 66, 88, 90, 124, 206, 212, 216, 245, 352, 369, 375, 393, 395, 518, 522, 544, 572). By analogy to EBV, differences between studies in the observed frequencies of detection of HHV-6 in specimens from HD patients might result from any of a variety of factors. Future studies of the role of HHV-6 in HD should be controlled for these factors.

A follow-up study by Dolcetti et al. (140) found that 3 (30%) of 10 specimens obtained from HIV-seropositive individuals with HD were PCR positive for HHV-6 DNA, compared to 13

(30%) of 43 tissue samples from HIV-seronegative patients with HD. HHV-6B DNA was identified in all 16 HHV-6-positive specimens; HHV-6A was detected in addition to HHV-6B in one of them. None of the specimens from HIV-infected hosts was HHV-6 DNA positive by blot hybridization, but all 10 had monoclonal EBV episomes detected by this technique. The investigators interpreted these data as being consistent with either normal HHV-6 infection of only a fraction of the EBV-positive malignant clone or with harboring of HHV-6 in normal infiltrating lymphocytes. Either possibility argues against a pathogenic role for HHV-6 in most cases of HIV-related HD.

No biopsy specimens were positive for HHV-6 DNA by blot hybridization in 51 patients with HD in three other investigations (246, 251, 492), although 9 (64%) of 14 specimens were HHV-6-positive by PCR in one series (type not specified) (492). The latter study also found HHV-6 DNA by PCR in 45 (59%) of 76 specimens from NHL patients, but none were positive by nucleic acid hybridization.

In considering the information described above, a number of lines of evidence suggest that HHV-6 may play an etiologic role in some patients with HD. (i) In some tumors, HHV-6 DNA is present in sufficient abundance to be detected by blot hybridization. This is far more abundant than EBV DNA that was detected in the same specimens. (ii) In the HHV-6-positive patients, RS cells constituted a greater proportion of cells in the tumor than in patients for whom specimens were negative for HHV-6 by blot hybridization. (iii) HHV-6 DNA has been found in RS cells of some tumor specimens by in situ hybridization. (iv) A significant fraction of specimens from HD patients are positive for HHV-6 DNA by PCR. (v) The relative rarity of RS cells in many specimens from HD patients may account for the detection of HHV-6 DNA by PCR but not by blot hybridization in some cases. (vi) HHV-6 DNA-positive tumor specimens are concentrated among patients with the nodular sclerosis type (and nodular sclerosis-lymphocyte depletion subtype) HD. (vii) Over half of the specimens positive for HHV-6 DNA by PCR in one study were negative for EBV DNA by the same technique. (viii) HHV-6 can infect some cells harboring EBV and influence EBV gene expression (5, 168), suggesting possible synergistic interactions in specimens from HD patients when both viruses have been detected. (ix) Some groups of HD patients have elevated anti-HHV-6 antibody titers, including some patients thought to have causative factors independent of EBV (discussed below). Although none of the accumulated evidence is definitive, HHV-6 remains a viable candidate as an etiologic agent in the pathogenesis of some HD cases, and further efforts to explore such a role for the virus(es) are warranted.

(iii) HHV-6 serology in lymphomas and leukemias. Antibody titers to HHV-6 in NHL or HD patients were not significantly different from or were moderately higher than those in normal subjects in two studies (307, 513). In one of these series, HHV-6 IFA titers rose in HD patients who relapsed posttherapy compared with patients who did not (307). Significant differences in HHV-6 seroprevalence and antibody titers between normal controls and patients with HD and low-grade NHL were found in another investigation (106). Young adults with HD and without siblings showed the greatest differences in rate of HHV-6 seroprevalence and geometric mean titer ratios compared to controls. Data presented above suggest that most NHLs are unlikely to be etiologically associated with HHV-6, despite the serologic data in this report. A recent study in the United Kingdom examined case clustering, EBV-RS cell status, and EBV and HHV-6 serologic findings (18). Elevated anti-HHV-6 titers were found primarily in patients with RS cells negative for EBV, suggesting an etiologic exposure for HD independent from EBV. The investigators hypothesized that the agent might be something other than HHV-6, because they previously failed to detect HHV-6 in biopsy specimens from HD patients by blot hybridization (246). HHV-6 should not be discarded as a candidate for involvement in HD on the basis of these results, since the virus was frequently detected in tissues from HD patients in several other studies performed by more sensitive methods. As is commonly the case, the serologic methods used in this study were not designed to distinguish between HHV-6 and HHV-7.

No significant differences were observed in the serologic response of patients with ALL in comparison with healthy controls (106, 306). HHV-6 antibody titers were elevated for acute myelogenous leukemia (AML) patients compared to healthy controls (106), but viral DNA was undetectable by blot hybridization in specimens from all 10 patients with AML in another series (251). Because HHV-6 might latently or persistently infect neoplastic cells in a manner that does not cause unusual expression of viral antigens, serologic data must be interpreted with caution when evaluating possible associations of HHV-6 and malignant disorders. Three examples of patients with HHV-6 integrated into PBMC DNA have been described (332, 334, 512). In each case, the anti-HHV-6 IgM titer was negative and the IgG titer was either negative or at a near-background level. One of these patients had nodular sclerosis-lymphocyte depletion subtype HD with tumor tissue that was blot hybridization positive for HHV-6; a second patient had a B-cell NHL.

(iv) Other lymphoproliferative disorders. Some other lymphoreticular neoplasms that are leukemias or have a leukemic phase have also been examined for a possible association with HHV-6. HHV-6 DNA was detected by hybridization in 14 (88%) of 16 patients with T-cell ALL (T-ALL) (326). HHV-6 DNA was detected in bone marrow lymphoblasts in all eight patients examined, with 20 to 58% of cells being positive. In addition, specimens from four of the eight patients were positive by IFA for expression of an early but not a late HHV-6 antigen. The pattern of viral gene expression in these cells could have been due to either latent or abortive infection of the T-ALL lymphoblasts.

A subsequent study of ALL involved 14 patients, 7 each with B-cell ALL (B-ALL) and T-ALL (48). Of the 14, 6 were children, 1 with B-ALL and 5 with T-ALL. Without nested PCR, HHV-6 DNA was detected in 4 (28%) of the 14 patients (all four had B-ALL) compared with 8 (26%) of 30 healthy blood donors. After nested PCR, 9 (64%) of the 14 patients and 15 (50%) of the 30 healthy blood donors were positive. The similar results in both populations led to the conclusion that HHV-6 is unlikely to play an etiologic role in ALL. Differences between the studies that may account for the different results include the use of bone marrow cells versus PBMC and differences in the study populations with respect to age distribution and form of leukemia. Thus, the question of a possible role for HHV-6 in T-ALL remains open.

HHV-6B DNA and virus-specific RNA were present in PBMC from two patients with $S100^+$ T-cell chronic lymphoproliferative disease (S100-CLPD), a very rare disorder (62). In the one case available for study, HHV-6 DNA was detected within the abnormal cells, which are characterized by expression of S100, a cytoplasmic, calcium-binding protein (207, 276).

Clinical associations: other neoplasms. (i) Kaposi's sarcoma. KS is a rare neoplasm originally described in elderly men of Mediterranean ethnicity (132). Four clinical categories of KS are now recognized: (i) classic or Mediterranean, (ii) African endemic, (iii) transplant associated or iatrogenic, and

(iv) epidemic or AIDS associated (reviewed in reference 386). In one study that looked for a possible association between HHV-6 and KS, 7 (35%) of 20 specimens from KS patients were positive for HHV-6 sequences by nested PCR (61). HHV-6-positive specimens were distributed among the KS types as follows: 4 (33%) of 12, African; 2 (29%) of 7, classic; and 1 (100%) of 1, AIDS associated. Of note, six (86%) of the seven positive specimens contained HHV-6A DNA, making this one of only a few studies of HHV-6 and disease association in which variant A was more prevalent than variant B. In a separate study with nonnested PCR, HHV-6 DNA was not detected in any of 22 patients with classic KS or in any of 4 patients with AIDS-associated KS (327). In a third study, 26 patients with AIDS were evaluated for the presence of HHV-6 DNA in biopsy or autopsy specimens of KS lesions (263). Of the 26, 9 (35%) were HHV-6 positive by PCR; all were HHV-6B. In two of four KS patients with HHV-6-positive biopsy specimens, HHV-6 DNA was also detected in the heart, lungs, liver, kidneys, and adrenals, suggesting no specific association with KS lesions. For comparison, dermatologic specimens obtained from 7 HIV-seropositive and 12 HIV-seronegative controls were positive in 6 and 2 patients, respectively (7 of the 8 positive specimens contained variant B). In addition, 7 (27%) of the 26 AIDS patients had HCMV sequences in the KS lesions, but none of the HIV-seropositive or HIV-seronegative controls did. These investigators concluded that the presence of HHV-6 and HCMV in AIDS-associated KS is most probably a result of disseminated viral infection rather than a specific association, although a cofactor role remains possible.

These investigations suggest that the ability to detect HHV-6 in KS lesions by PCR may be very dependent on methodology, with positive results more likely to be obtained with nested than with nonnested PCR. No etiologic association of HHV-6 with KS can be inferred from these studies.

A previously unidentified herpesvirus was identified in KS lesions by using representational difference analysis (91, 367); the virus is known as HHV-8 or Kaposi's sarcoma-associated herpesvirus. HHV-8 has been detected in specimens from patients with all four forms of KS (20, 91, 151, 367, 465), as well as in tissues or cells from patients with some other neoplasms (91, 256, 444, 526). Rapidly accumulating molecular (20, 91, 231, 367, 465) and seroepidemiologic (186, 187, 259, 305) evidence is consistent with HHV-8 playing an etiologic role in KS.

(ii) Oral carcinoma. In a recent study, HHV-6 DNA and an HHV-6 glycoprotein (gp116K/64K/54K) were detected in oral carcinoma tissue specimens by PCR and IHC, respectively (548). Of fresh tissue specimens taken from nine patients from India with squamous cell carcinoma of the buccal mucosa, six (67%) were positive for HHV-6 DNA. HHV-6 DNA was detected in five (71%) of seven other patients. For all seven samples, gp116K/64K/54K was detected in the cytoplasm of squamous cells as well as in membranes and nuclei on occasion. No staining was seen in oral mucosa samples from healthy controls and from several patients with nasopharyngeal carcinoma, and no PCR amplification was detected when EBV-specific primers were used. Five of five patients with late-stage oral carcinoma had elevated HHV-6-specific IgA titers, while five age-matched healthy controls were negative. Of note, patients with EBV-associated nasopharyngeal carcinoma often have very elevated IgA titers to various EBV antigens (377, 521, 575).

Oral carcinoma is common in Asia and has been associated with the widespread use of chewing tobacco and other substances that contain chemical carcinogens (253). It is possible that HHV-6 in a replicative state is specifically associated with oral carcinoma, possibly as a cofactor, and further investigation of a larger patient population is warranted.

(iii) Cervical carcinoma. In a follow-up to in vitro studies with cervical carcinoma cell lines, Chen et al. examined fresh surgical or paraffin-embedded biopsy specimens from 72 women with squamous cervical carcinoma (SCC) or cervical intraepithelial neoplasia grade III (CIN III) (95). HHV-6 DNA was detected in specimens from 6 (8%) of the 72 patients by nested PCR followed by blot hybridization. In contrast, no cervical specimens obtained from 30 patients with nonmalignant conditions were HHV-6-positive by the same technique. HPV DNA was detected by PCR in specimens from 58 (85%) of the 72 patients; of these, 47 (81%) had DNA of HPV-16, a virus strongly associated with anogenital carcinomas (576). Specimens from four of the six HHV-6-positive patients were also positive for HPV-16 DNA. ISH analysis of four HHV-6-positive specimens revealed HHV-6 DNA in occasional nuclei of epithelial cells. This finding contrasts with the pattern of widespread distribution of viral DNA in typical HPV-16-positive SCC or CIN III lesions. The ISH results are compatible with HHV-6 acting by a hit-and-run mechanism as a cofactor with HPV in causing some cases of cervical carcinoma or with HHV-6 occasionally being a passenger in transformed cervical cells. Another explanation for the small number of positive cases is that HHV-6 is occasionally a passenger in transformed cervical epithelial cells. Further work is required to distinguish between these and other possibilities.

(iv) Conclusions on the role of HHV-6 in malignancy. A possible role has been postulated for HHV-6 in a variety of neoplastic disorders, but as yet there has been no conclusive demonstration that either HHV-6A or HHV-6B is involved in any causative way with any malignancy. Nevertheless, the accumulated data suggest that at least for some neoplasms, there is more than a chance association with the virus(es). A summary of the relationship between HHV-6 and neoplasia is provided in Table 5.

Chronic Fatigue Syndrome

In recent years, there has been great interest among both physicians and the general public in the chronic fatigue syndrome (CFS), a condition characterized by prolonged or chronic fatigue and a combination of four or more of the following symptoms that are concurrently present for at least 6 months: impaired memory or concentration, sore throat, tender cervical or axillary lymph nodes, muscle pain, multijoint pain, new headaches, unrefreshing sleep, and postexertion malaise (184). CFS can be diagnosed only in the absence of other known medical or psychiatric causes of fatigue, and no specific physical findings or laboratory tests are diagnostic. Because of these features, some investigators believe that CFS is composed of a group of heterogeneous disorders rather than being a single disease with a specific etiology (125, 184).

Despite the vagueness of the condition, several hypotheses have been advocated to explain the pathophysiology of CFS. According to one view, CFS results from psychological sequelae of an acute infection in an emotionally susceptible person; this hypothesis is based on studies indicating that well-recognized psychiatric disorders occur in a majority of patients with CFS (125). Another hypothesis is that CFS is due to a persistent immune system dysfunction induced by an infectious agent (125, 353). Although abnormalities in the immune response to various viral agents and nonspecific indicators of inflammation, such as antinuclear antibodies, have been noted, no definitive evidence has emerged which demonstrates that immune dysfunction following infection is either causative or a

TABLE 5. HHV-6 and neoplasia

Neoplasm	HHV-6 Relationship	Comment
NHL	Low proportion of PCR-positive samples in most studies. A few specimens positive by blot hybridization. Scattered cells or small clusters positive for HHV-6 markers in several cases.	Clonality of NHLs weighs against etiologic association for most HHV-6-positive cases.
HD	12 to 30% of samples positive by PCR in some studies. A few specimens positive by blot hybridization. Positive specimens tend to be from nodular sclerosis type of HD. Presence of HHV-6 antigens in RS cells in one study.	PCR-positive specimens potentially more meaningful than with NHLs, due to relative rarity of RS cells. HHV-6 remains a candidate for etiologic role in some cases.
ALL	14 of 16 T-ALL patients positive by blot hybridization with peripheral cells in one study. 0 of 7 T-ALL and 4 of 7 B-ALL positive by nonnested PCR in second study. Similar rate of positives (all patients) compared to controls.	Disparate results may be due to difference in patient populations or methods used. Specific HHV-6 association with T-ALL not ruled out.
S100-CLPD	2 of 2 patients positive by blot hybridization. Viral genome in S100-CLPD cells in one case tested.	Specific HHV-6 association possible.
KS	35% of specimens positive by nested PCR in two studies. Positive results may be due to viral dissemination to skin.	No HHV-6 etiologic association shown. HHV-8 is a better candidate as causative agent.
Oral carcinoma	67 and 71% of buccal squamous cell carcinomas positive by PCR in a small series. Viral glycoprotein detected in 100% of 7 samples in second series. HHV-6-specific IgA elevated in 100% of 5 patients, 0% of 5 matched controls.	Data suggest possible association with replicating HHV-6
Cervical carcinoma	Only 8% of specimens positive by nested PCR, compared to 81% for HPV. HHV-6 DNA in occasional nuclei in 4 PCR-positive specimens examined.	Little to suggest a specific association.

marker for the development of CFS. An alternative explanation for CFS has recently been proposed which views the condition as resulting from a disorder of the hypothalamic-pituitary-adrenal axis and is precipitated by an emotional or physical stress, such as infection (125). Hypothalamic-pituitary-adrenal axis abnormalities are associated with disturbances of mood and sleep, as well as with immune dysfunction, thus accounting for prominent features noted with other explanations. A common factor among these hypotheses for the pathophysiology of CFS is a frequent association with acute infection as a precipitating event for the syndrome.

Over the past decade, various viral agents, including EBV, HSV, HCMV, enteroviruses, and a retrovirus have been scrutinized for a possible specific relationship with CFS; no firm or consistent relationship had been identified for any of these agents. At the time of the initial description of HHV-6 (450), an apparent outbreak of CFS was reported from Lake Tahoe, Nev. (143). Although suggestions linking HHV-6 to this outbreak were later proven unfounded (308), speculation has continued that the virus is etiologically related to at least some cases of the syndrome. Studies of HHV-6 seroprevalence and antibody titers in patients and controls have provided mixed results (5, 43, 71, 121, 194, 308, 349, 428, 447, 527). Evidence for increased HHV-6 replication has been reported in patients with various long-term illnesses (70), but this study was criticized for shortcomings in criteria for patient selection, laboratory methods, and statistical analysis (429).

Several reports are of particular interest with regard to HHV-6 and CFS. HHV-6 DNA was detected in PBMC from 7 (54%) of 13 CFS patients and 0 of 13 healthy controls by PCR with variant-specific primers (551). Of the seven HHV-6 positive patients, 4 (57%) had HHV-6A. HHV-6 was not detected by culture of PBMC from either CFS patients or matched controls. Seroprevalence to an early antigen and antibody titer to a late antigen of HHV-6 were increased in the patient group relative to controls, with similar results regardless of which HHV-6 variant was used as the antigen source. In another study, PBMC of CFS patients and healthy controls were assayed for DNA of HHV-6 and HHV-7 by variant-specific PCR (139). HHV-7 DNA was detected in over 80% of both CFS patients and controls, and the proportion of HHV-6B-positive specimens was similar in both groups. However, HHV-6A DNA was detected in 8 (22%) of 36 CFS patients and in only 1 (4%) of 24 controls ($P = 0.05$). These two studies are among only a few to find a possible association of HHV-6A with a clinical disorder. Patnaik et al. (402) examined the sera of CFS patients who met the most recent case definition of the syndrome (184) and healthy controls for antibodies to HHV-6 early antigen (EA; p41/38). Of 154 CFS patients, 61 (40%) had IgG to EA, compared with 13 (8%) of 165 controls. In addition, 93 (60%) of 154 CFS patients had IgM to EA, in contrast to only 7 (4%) of the 165 controls. The differences for both IgG and IgM were statistically significant.

These studies are of interest in that they suggest that some CFS patients have atypical responses to HHV-6, possibly indicative of viral activity. However, no diagnostically useful cutoff was identified, and it is impossible to assess the significance of these observations in the absence of information about the patterns of reactivity with these antigens in patients with other diseases or immune disturbances. As noted by these investigators, these observations are consistent with reactivation of latent infection due to immune dysregulation but do not prove that HHV-6 is the cause of the symptoms. The data presented in these reports should be viewed in the context of (i) investi-

gations, including some cited above (194, 222, 447), which have shown elevated antibody titers to different members of the herpesvirus family, as well as to other viral agents such as measles virus (222), which can persistently infect their hosts; and (ii) the level of HHV-6 activity being insufficient to allow culture of infectious virus (551). Further work is required to determine whether HHV-6 contributes to the clinical manifestations or whether the response to the virus reflects the underlying pathologic mechanisms of this complex and often frustrating (to both patients and physicians) syndrome.

Collagen Vascular Diseases

HHV-6 activity in collagen vascular diseases has been studied; the available data do not support an etiologic association between the virus and these diseases. The relevant studies are summarized here. A cohort of 25 women with primary Sjögren's syndrome had no significant differences in HHV-6 seroprevalence or antibody titers compared with a group of 25 age-matched healthy women (38). In another study, 49 patients with primary Sjögren's syndrome and 50 age- and sex-matched normal controls had similar rates of HHV-6 seroprevalence or prevalence of HHV-6 DNA detected by PCR in PBMCs or salivary gland biopsy specimens (424). HHV-6 antibody titers in 25 women with rheumatoid arthritis were either lower or slightly higher than in an equal number of age- and sex-matched healthy controls, when measured by IFA or enzyme-linked immunosorbent assay, respectively (38). HHV-6 has been isolated from peripheral blood lymphocytes of several patients with various collagen vascular diseases, most often from patients with systemic lupus erythematosus (295).

TREATMENT

HHV-6 has been tested for susceptibility to various antiviral agents in vitro (reviewed in reference 543). Most work in cell culture has involved inhibitors of herpesvirus DNA polymerases, such as phosphonoacetic acid (134, 463), phosphonoformate or foscarnet (10, 74, 481), acyclovir (9, 12, 46, 74, 134, 267, 441, 481), and ganciclovir (9, 12, 74, 441). The results of these investigations are not directly comparable because of differences in the assays, cells, and virus strains used. Despite these limitations, the data indicate greater susceptibility of HHV-6B to ganciclovir and foscarnet than to acyclovir, which inhibited viral replication only at high concentrations. This pattern resembles that for HCMV. The susceptibility of HHV-6A to acyclovir and foscarnet is similar to that for HHV-6B. However, in some reports, some HHV-6A strains (SIE and TAN) were susceptible to ganciclovir (9, 12), while in others, HHV-6A(GS) was relatively resistant (14, 481). Foscarnet may thus be preferable to ganciclovir for anti-HHV-6 therapy in situations where the variant or susceptibility are not known. Phosphonoacetic acid is also inhibitory to HHV-6B(Z29) (74, 134, 463), but its activity with regard to other HHV-6 isolates has not been studied. The corticosteroids hydrocortisone and dexamethasone have shown both enhancement and suppression, respectively, of HHV-6 replication in vitro (58, 442).

Only a few reports have described antiviral use in humans for treatment of illness thought to be associated with HHV-6, and all of them described uncontrolled studies. HHV-6 viremia resolved in four BMT recipients treated with ganciclovir (146). Ganciclovir therapy was associated with resolution of renal allograft rejection in a patient with elevated HHV-6 IgM titers and no evidence of HCMV replication (242). Among 20 BMT recipients treated with antiviral agents for HCMV viremia, 7 also had HHV-6 viremia as determined by PCR of PBMC. Of these, only one (25%) of four treated with ganciclovir lost detectable HHV-6 sequences in PBMC, but all three (100%) of those who received foscarnet became HHV-6 DNA negative after treatment (528).

The efficacy of antiviral drugs in presumed HHV-6 infection has not been evaluated in controlled trials. However, obtaining definitive data proving a significant antiviral effect for agents commonly in use today may be difficult for the following reasons. (i) The least toxic antiherpes drug, acyclovir, has little in vitro effect on HHV-6 when used at clinically achievable concentrations. It is interesting that in spite of this, BMT patients who received acyclovir for other reasons were less likely than others to have HHV-6 activity during the posttransplantation period (254). (ii) Children with primary infections, the patient population most likely to yield unequivocal results, would not be considered good candidates for agents such as ganciclovir or foscarnet because of the generally benign nature of their illness and the toxicities associated with these drugs. The applicability of data obtained in such a population to other clinical situations is uncertain. (iii) Immunocompromised hosts, such as renal transplant or BMT recipients or persons with advanced AIDS, frequently have complex illness with multiple pathogens detected at various body sites. Other potential pathogens that might be encountered, such as HSV or HCMV, may also be inhibited by antiviral therapy, making evaluation of the response observed difficult with respect to one particular infectious agent. Concomitant therapy with antimicrobial agents used for common nonviral pathogens can add to the uncertainty regarding the effect of therapy directed at HHV-6. Furthermore, the etiologic relationship of HHV-6 to specific end-organ disease in such hosts is unclear.

Despite these potential difficulties, we have identified three situations in which prospective evaluation of antiviral therapy for HHV-6 infections should be considered: transplant recipients with idiopathic pneumonitis, MS patients, and patients with HHV-6-associated encephalitis. Appropriate longitudinal, well-controlled, prospective trials of anti-HHV-6 chemotherapy will require coordinated multicenter efforts to obtain sufficient patients for statistical analysis, as well as the ability of participating centers to provide extensive laboratory support, including techniques such as HHV-6 culturing, serologic analysis, quantitative PCR, and IHC staining. Even then, the results obtained may not be definitive, given the complexity of disease in these patients.

MS patients may have illness with sufficient morbidity and mortality to warrant the use of presently available toxic antiviral agents. Study arms could use currently available but noncurative therapy, with or without the addition of an antiviral agent. Foscarnet may be superior to ganciclovir as a chemotherapeutic agent for such a study, since it can inhibit the growth of both variants and more reliably attains levels in the CSF sufficient for in vitro anti-HHV-6 activity (10, 74, 215). Retrospective study of MS patients who were treated with antiviral agents for HCMV or HSV infections might provide another source of useful information, although this would not replace a well-controlled, prospective trial. Empirical anti-HHV-6 therapy is not justified in MS patients.

Development of a potent HHV-6 antiviral agent which is much less toxic than those presently available would obviously enhance the ability to evaluate the treatment of HHV-6-associated illness.

Finally, an apparently immunocompetent man whose lungs were dually infected with *L. pneumophila* and HHV-6 was treated for an extended period with the usual antimicrobial therapy for *L. pneumophila* (442). The patient responded only when high-dose methylprednisolone was given. HHV-6 recov-

ered from his blood was inhibited in vitro by dexamethasone. It is unknown whether his recovery was due to an antiviral effect of the drug, suppression of immunopathology by the drug, or unrelated coincidence. Given the potentially deleterious immunosuppressive effects of systemic corticosteroids in patients with many infections, we believe further in vitro studies to confirm these results are warranted but agree with the clinical use of such agents only in similar situations in which other forms of therapy have been unsuccessful for gravely ill patients.

IMMUNE RESPONSE

Most neonates are protected from primary infection with HHV-6 by the presence of virus-specific antibody of maternal source (64, 208, 229, 278, 392, 568, 569). Titers of antibody to HHV-6 have been reported to be elevated in umbilical cord blood relative to those in maternal blood (568, 569). By the age of 3 to 5 months, however, both seroprevalence and the geometric mean titer of anti-HHV-6 antibody reach a nadir (46, 64, 208, 278, 392, 568, 569). As maternal antibody wanes, the rate of primary infection rises, with peak rates of infection noted between 6 and 12 months of age (208, 229). The viremia observed during primary infection subsides with the appearance of neutralizing antibodies of the infected child (27). As expected, the earliest class of neutralizing antibody is IgM, first observed 5 to 7 days after the onset of clinical manifestations (484). The highest titers of IgM are seen at 2 to 3 weeks after infection, and IgM is usually undetectable by 2 months postinfection (484). Anti-HHV-6 IgG usually appears within 7 to 10 days after fever has subsided (41, 519), increases in avidity over time (531), and remains at measurable levels for many years. HHV-6-specific IgA titers were elevated in the limited number of patients with advanced oral carcinoma examined (548).

Differences in antibody titer to HHV-6A and HHV-6B in serum have been evaluated in limited studies. One investigation observed IFA titer differences to variants A and B of greater than 2 dilutions in nearly 5% of serum samples collected from 234 Malaysians of diverse ethnicity (549). Another study noted similar IFA titers to HHV-6A and HHV-6B in 81% of samples from 136 adults in the United States, with 19% showing two- to fourfold differences in titer (89). These results may reflect differences in serum antibody cross-reactivity to polypeptides common to the two variants or differences in antibody response to polypeptides unique to either variant, or both. Also, persons infected with both HHV-6A and HHV-6B may express a humoral response which may be dependent upon the relative burden of primary or reactivated infection with the two variants.

In vitro, T-cell clones with activity against HHV-6 have been described (559). Clinical data indicate that a cellular immune function important for control of either primary or reactivated HHV-6 infection is depressed in immunocompromised hosts. This would include HHV-6 viremia (566) or increases in antibody titer after renal transplantation (359, 388, 566); the culture of HHV-6 from blood, bone marrow, and BAL or oral lavage specimens (79, 81, 146, 254, 539); and elevated levels of HHV-6 genomes by PCR (110) in BMT patients, as well as the evidence for widespread dissemination of HHV-6 in various tissues of AIDS patients (116, 281). Induction of IFN-α by in vitro exposure of PBMC and cord blood mononuclear cells to HHV-6 suggests that this natural antiviral substance may play a role in limitation of HHV-6 infection in vivo (270). IFN-α induction was enhanced in PBMC relative to cord blood mononuclear cells and was observed with UV-irradiated as well as infectious HHV-6, indicating that infection is not required for the response. Exogenous IFN-α suppresses HHV-6 replication in vitro (270, 496). HHV-6 infection of PBMC in vitro induces up-regulation of NK cell cytotoxicity, an effect that is greatly diminished by MAb to IL-15 (169). HHV-6 replication is also significantly reduced in PBMC cultures containing exogenous IL-15. This suggests that IL-15 may be important for activation of host defenses, including enhancement of NK cell activity, for control of HHV-6 infections. Both enhanced NK cell activity and elevated levels of IFN-α in the circulation occur during acute primary infection relative to convalescence, providing further support for the control of HHV-6 infection by nonspecific components of the immune response (496).

Since HHV-6 can infect cells such as lymphocytes, macrophages, and NK cells, all of which play vital roles in the immune system, it has been postulated that the virus is immunosuppressive. Effects of HHV-6 infection on bone marrow progenitor cells were described above. HHV-6A infection of T-cell clones in vitro resulted in decreased expression of surface CD3 (185, 340). Evidence favoring either transcriptional (340) or posttranscriptional down-regulation (185) has been reported. The investigation supporting modulation at the transcriptional level found that decreased CD3 expression required viral DNA replication and that surface αβ T-cell receptor expression was also reduced. A similar down-regulation of surface CD3 in vivo may prevent T-cell activation. No significant loss of surface CD3 was observed in experiments with HHV-6B (185). In vitro, both HHV-6A and HHV-6B inhibit the lymphoproliferative responses of PBMC to mitogen stimulation (166, 223), an effect which occurs with UV-inactivated virus and thus is likely to be mediated via a virion protein(s) (166, 223). Infection by HHV-6A of PBMC or T-cell-enriched cultures suppressed IL-2 synthesis after mitogen stimulation (166). Similar effects were achieved with UV-irradiated virus. Levels of IL-2 transcripts were diminished in experiments with enriched T-cell cultures or Jurkat cells (166). In separate work, HHV-6A greatly reduced the respiratory burst capacity of peripheral blood monocytes in response to phorbol myristate acetate but not to opsonized zymosan, suggesting selective interference with monocyte activation by the protein kinase C pathway (72).

The hypothesis that HHV-6 and HIV-1 are synergistically immunosuppressive has received much attention. HHV-6 and HIV-1 can dually infect $CD4^+$ lymphocytes in vitro (336, 385), and HHV-6A can induce CD4 expression in $CD4^-/CD8^+$ lymphocytes (185, 335), γ/δ lymphocytes (339), and NK cells (341), thus facilitating the entry of HIV-1 into cell subpopulations that are otherwise refractory to that virus. This suggests a mechanism by which HHV-6 might expand the pool of HIV-1-susceptible cells in vivo. The effects of HHV-6 and HIV-1 coinfection in cell culture remain controversial, as described in the section on HHV-6 and HIV-1 interactions, above. How this translates into effects in the HIV-1-seropositive host is even less clear. Clarification of the relevance to human biology is needed with respect to observed in vitro effects of HHV-6 on induction of soluble factors such as IFN-α, TNF-α, and IL-1β, as well as on inhibition of IL-2 expression and growth suppression of cellular populations such as lymphocytes, monocytes, macrophages, and bone marrow precursor cells.

Concurrent infections of HHV-6 with other human pathogens including measles virus, HCMV, HSV-1, EBV, dengue virus, adenovirus, coxsackie B virus, parvovirus B19, *L. pneumophila*, *P. carinii*, and possibly HHV-7 have been reported (41, 102, 238, 286, 412, 442, 487). Whether these concurrent infections with HHV-6 are specifically associated with an immunosuppressive effect or are coincidental has not been determined.

PERSISTENCE AND LATENCY

At the cellular level, viral infections can be lytic, latent, or abortive. Infectious viral progeny are produced during lytic infection, and host cells are normally killed in the process. During latent infection, infectious progeny are not produced and viral gene expression is limited to the genes required for maintenance of latency. Abortive infections occur when the host cell permits viral entry but cannot sustain a complete lytic cycle. Latently infected cells can be considered to be abortively infected cells that retain the virus in a form that can proceed to the lytic cycle in response to changes in the cellular environment. Persistent infection is a consequence of achieving a balance between lytic infection-induced cell death and cellular replication; it is not to be confused with latency. In vivo, it can be difficult to distinguish these states. The terms "latency" and "persistence" are frequently used with different definitions in reference to clinical infections. Thus, clinical latency is the period both between primary infection and disease and between periods of obvious viral activity, and persistence means that the virus is resident in the host for an extended period, possibly for the remainder of the host's life, without regard to the cellular mechanism of the persistence.

The previously identified human herpesviruses all persistently infect their hosts following primary infection. Latent viral infection, characterized by a lack of lytic viral replication in transiently nonpermissive cells, occurs in such diverse sites as trigeminal ganglia (HSV-1), thoracic dorsal roots (VZV), and circulating B lymphocytes (EBV). Reactivation, with replication of infectious virus, may occur at these sites, leading to recurrent disease. It is thus natural to expect that HHV-6 persistently infects humans after primary acquisition and that it might persist in some location in a truly latent form.

There are multiple lines of evidence to support the concept of HHV-6 persistence in the host, but less data to convincingly show that it establishes cellular latent infection. The evidence to support persistence includes the following.

Immune Response

The presence of HHV-6 IgM in approximately 5% of the adult population without recent or concomitant illness suggests that HHV-6 replicates in a subclinical manner in persons who were previously infected, although reinfection with another strain of HHV-6 has not been ruled out (484). Significant increases in IgG titers in immunocompromised hosts, such as those with renal allograft transplantation (359, 388, 566), as well as elevated serologic titers in certain populations with neoplastic disorders, such as HD or NHL, when compared with healthy controls (307, 513) are also consistent with reactivation of HHV-6. Of children monitored 1 to 2 years after primary HHV-6 infection, 26% had a second fourfold or greater rise in HHV-6 serologic titers, suggestive of either reactivation or reinfection with a second strain (208). As noted above, the degree to which cross-reacting antibodies to HHV-7 may have altered the results of these serologic studies is unclear, although it is likely that the serologic response detected in most investigations was due predominantly to HHV-6 infection. Rises in HHV-6 antibody titers have been detected in hosts who have had primary infections with another HHV. Solid organ transplant recipients have increases in non-cross-absorbable HHV-6 antibodies when infected by HCMV (533) and in high-affinity antibodies to HHV-6 during infection by HCMV or EBV (532, 533). HHV-6 titers also rose in patients who experienced ES in association with HHV-7 infection after primary infection with HHV-6 (217, 515, 520). These data are consistent with reactivation of HHV-6 in those undergoing infection with another herpesvirus. The ability of HHV-7 to activate lytic replication of HHV-6B in cultured lymphocytes was described in an elegant series of experiments (258); it remains to be seen whether this effect can take place in vivo and is a source of the increased HHV-6 antibody titers associated with HHV-7 infection. Antibody cross-reactivity may also explain the boost in HHV-6 antibody titer upon infection of humans with HHV-7 (217, 520), although the data do not unambiguously distinguish this possibility from reactivation. The two possibilities are not mutually exclusive.

Detection in Blood

Reported rates of HHV-6 PCR positivity in PBMC of healthy blood donors range from approximately 5 to 90% (112, 119, 160, 189, 201, 243, 331, 401, 541). Differences in the observed rates are probably due to the quantity of purified DNA or cell extract used as a target, as well as variation in the PCR techniques used. Nevertheless, a large proportion of healthy individuals harbor HHV-6 DNA within PBMC, indicative of viral persistence. In the study by Hall et al. (208), 7% of children with acute HHV-6 infection had sequential HHV-6 PCR status change from positive to negative to positive in PBMC over a 1- to 2-year follow-up period, suggestive of either reactivation or reinfection. The finding of an identical HHV-6 DNA restriction fragment pattern in peripheral lymphocytes obtained from the blood of a BMT patient both before and after transplantation argues for persistent infection rather than reinfection with a new strain of HHV-6 (564). HHV-6 DNA has been identified by PCR in lymphocytes during primary infection and later in macrophages during convalescence or in healthy adults, indicating possible localization within macrophages as a site of persistent or latent infection (288). Treatment of macrophages with 12-*O*-tetradecanoylophorbol 13-acetate led to productive infection in cell culture, consistent with reactivation from latency. In addition to chemical induction, HHV-6B can be induced to lytic replication by cocultivation of primary PBMCs with HHV-7-infected cells (258). The pathogenic effects of this interaction may prove to be important.

Presence in Saliva and Salivary Glands

HHV-6 has unambiguously been isolated from saliva (55, 312, 374), and its DNA has been detected in saliva (112, 136, 503) and salivary glands (424) of healthy persons. In addition, HHV-6 has been detected by IHC staining and ISH in tissue sections from healthy salivary glands (174, 296) and healthy-appearing areas of salivary glands containing benign tumors (174). However, questions have been raised about the specificity of the probes used in the latter studies because, as with the virus isolation studies described above, they were done prior to the discovery of HHV-7 and the recognition that some reagents are cross-reactive between HHV-6 and HHV-7 (57).

Detection in CSF and Brain

A high proportion of children with past HHV-6 infection retain HHV-6 DNA in CSF, suggesting that the CNS may be a reservoir for persistent infection (84). Consistent with this hypothesis, HHV-6-specific DNA was detected by PCR in over 80% of autopsy brain specimens, suggesting that the CNS of most people is persistently infected with HHV-6 (87, 329, 330). Viral antigens were detected in astrocytes, brain macrophages, and gray matter neurons (87). These studies point to persistent infection of the CNS by HHV-6 of many persons long after primary infection.

Isolation from and Detection in Renal Biopsy Specimens

Viruses which were presumably HHV-6 were isolated from 3 (5%) of 58 intraoperative renal transplant biopsy specimens (566). The viruses were identified in cell culture by IFA with antiserum from a child with ES. In another study, HHV-6 antigens were detected by IHC staining with MAb in 3 (50%) of 6 intraoperative specimens and 11 (50%) of 22 biopsy specimens (226). Since the viral antigens were detected in the absence of viremia, these reports suggest that the kidneys may harbor HHV-6 persistently or latently.

HHV-6 DNA in Lungs

The lungs may also be a site of HHV-6 latent or persistent infection, as evidenced by the frequent finding of HHV-6A and/or HHV-6B DNA by PCR in pulmonary specimens (110, 115). Alveolar macrophages could conceivably harbor HHV-6 in this location. HCMV can also persistently infect lung tissue (45).

Presence in Genital Tract

HHV-6 DNA was detected by PCR more frequently in the cervices of pregnant women than in nonpregnant controls, suggesting reactivation of HHV-6 in the genital tract during pregnancy (390). In addition, HHV-6 DNA was detected in vaginal secretions of women who visited a sexually transmitted diseases clinic (303).

Identification in Neoplastic Cells and Tissues

HHV-6 DNA and antigens have been detected in neoplastic cells and tissues from patients with a number of malignancies, including NHL (60, 133, 175, 246, 251, 333, 560), HD (133, 293, 513), T-ALL (326), S100-CLPD (62), KS (61, 262), oral carcinoma (548), and cervical carcinoma (94). Although the etiologic relationship of HHV-6 with these conditions is uncertain, their occurrence long after most persons have had primary HHV-6 infection and the chronic nature of most of these conditions suggest that HHV-6 can persistently or latently infect these neoplastic tissues. Of further interest, HHV-6 DNA has been detected in an apparently integrated form in the chromosomal 17p13 locus of PBMC from three patients with either non-AIDS-related NHL, HD, or MS (332, 334, 512). It is unclear whether this integration is indicative of a latent form in healthy hosts, from which productive infection may be reactivated.

The body of evidence cited above argues strongly for persistent subclinical infection with HHV-6 following primary exposure in most persons. To date, no cell lines in vitro have been developed in which HHV-6 remains in a latent state, without viral replication. Whether HHV-6 establishes a true latent, as opposed to persistent, infection in humans remains undetermined.

ANIMAL MODELS

The development of an experimental animal model for HHV-6 would provide a system in which viral pathogenicity, latency, reactivation, and therapeutic methods could be studied.

Viruses similar to HHV-6 have been assayed for in several nonhuman primate species by serologic and PCR methods. Higashi et al. tested a total of 215 serum specimens from Old World and New World monkeys belonging to 10 different species and found HHV-6-reactive antibodies in monkeys from 8 of the 10 species tested (218). This suggested the existence of HHV-6 or a closely related virus in at least some monkey species. However, in another study, serum specimens from 38 Old World primates and 8 New World primates were tested, and none were positive for HHV-6 antibodies (342). The primate species tested in common in these two studies were African green monkeys (*Cercopithecus aethiops*) and chimpanzees (*Pan troglodytes*); 13 (81%) of 16 *C. aethiops* and 24 (80%) of 30 *P. troglodytes* animals were HHV-6 antibody positive in the study by Higashi et al. In another study, HHV-6 DNA was not detected by PCR in PBMC of pig-tailed macaques (*Macaca nemestrina*) (344), suggesting that these monkeys are not commonly infected by a virus closely related to HHV-6.

Although in the initial description of HHV-6, no nonhuman cell lines that would support propagation of the virus were identified (450), in more recent studies, PBMC from nonhuman primate species were susceptible to in vitro infection by HHV-6A and HHV-6B. Serum from ES patients was able to transmit febrile illness with transient lymphocytopenia to rhesus macaques (*Macaca mulatta*) (261), and the illness could be propagated to other monkeys. These experiments were done nearly four decades before the discovery of HHV-6, and the infectious agent in the ES patient serum was not identified, although it is likely that it was HHV-6. Chimpanzee and pig-tailed macaque PBMC can be productively infected by HHV-6A and HHV-6B (312, 342, 344), while HHV-6B can also replicate in vitro and in vivo in African green monkeys and cynomolgus macaques (*Macaca fascicularis*) (552). Chimpanzee T lymphocytes can be simultaneously infected with HHV-6 and HIV-1 (342). Two-color indirect IFA was used to demonstrate that antigens specific for both HHV-6 and HIV-1 were expressed in individual cells. In addition, an accelerated cytopathicity was observed in dually infected cultures compared to cultures infected with either HHV-6 or HIV-1 alone. Lymphocytes from *M. nemestrina* can be productively infected with either of the HHV-6 variants and simian immunodeficiency virus (SIV); this can lead to an increase in SIV replication in dually infected cells (344). *M. nemestrina* develops an AIDS-like condition following SIV infection and therefore may provide a useful animal model for studies of in vivo interactions between HHV-6 and a virus that leads to an AIDS-like condition.

SCID mice in which with human fetal tissues were implanted (SCID-hu) may provide a useful animal model to study the susceptibility of different type of human tissues to HHV-6 infection. This system has been successfully used to localize HCMV replication to epithelial cells in human thymus and liver implants (365). The use of SCID-hu mice may also facilitate the isolation of clinical HHV-6 isolates and provide a model that will allow dissection of various aspects of the host-virus interaction. Human hematopoietic tissue implants might also be used to study tissue-specific gene expression between HHV-6A and HHV-6B. Differences in either growth or in gene expression may aid in understanding the biological differences between HHV-6A and HHV-6B.

DIAGNOSIS

Methods have been developed for the detection of infectious virus and its antigens and nucleic acids, as well as for the detection of antibodies against the virus. Interpretation of the results of diagnostic assays for HHV-6 is complicated by the high seroprevalence and its constitutive presence in blood. Convenient and sensitive assays that can readily discriminate active from latent infection need to be developed. Some assays demonstrate significant associations between a given viral

marker and whatever disease is being studied. This ability is useful for studying populations, but activity cutoffs that would allow a definitive association between HHV-6 and disease in an individual have seldom been defined. The diagnostician must be ever mindful that proximity of the virus to disease-related tissue does not prove an etiologic association.

Virus Detection

Culture. HHV-6 can be cultured from the lymphocytes of a high percentage of children during the febrile, prerash phase of ES (26, 31, 387, 497, 556). This has been done by culturing patient lymphocytes either alone or with other fresh lymphocytes. Lymphocyte activation with either phytohemagglutinin or antibody to CD3 and maintenance in IL-2 are required. With the exception of the febrile, prerash phase of ES, HHV-6 is only infrequently detected by culture methods, although some isolates have been obtained during periods of immunodeficiency such as during AIDS or transplant-associated immunosuppression. ES is the only clinical entity where HHV-6 isolation plus the clinical picture allow a nearly definitive diagnosis. It is more difficult to interpret positive cultures obtained during periods of immunodeficiency because viral activity may be unrelated to obvious clinical events.

Antigens. HHV-6 antigens can be detected with any of an array of MAb (42, 76, 89, 170, 241, 391, 553), some of which are commercially available. Some of the MAb react with proteins specified by both HHV-6A and HHV-6B, while others are variant specific (summarized in reference 170). In addition to IFA, some of the MAb are suitable for use on fixed tissue sections. Antigen detection is useful for culture confirmation and detection of viral proteins in tissues. A commercial antigen capture EIA based on gp116/64/54 was recently described that detects antigens from both HHV-6 variants (348). The sensitivity of the assay was similar to virus detection by culture from plasma obtained from children with ES; therefore, the assay is worth evaluating for its utility in rapid differential diagnosis of febrile children and for monitoring HHV-6 activity in transplant recipients.

Nucleic acids. HHV-6 nucleic acids can be detected by hybridization and by PCR. Southern blot hybridizations can be useful for rapid screening of large numbers of specimens. It is generally a less sensitive technique than PCR. This has potential advantages as well as disadvantages. On the positive side, positive results will seldom be due to simple background HHV-6 activity. On the negative side, its low sensitivity can obviously lead to incorrect conclusions about the presence of viral nucleic acids. Numerous PCR primer sets that are sensitive and specific have been described for HHV-6 (28, 34, 35, 37, 101, 109, 110, 112, 227, 265, 277, 287, 289, 356, 417, 457, 458, 554); some of these sets allow easy discrimination of the variants (37, 101, 554). The presence of HHV-6 in the saliva of laboratory personnel can lead to false-positive PCR results if appropriate precautions are not taken (111, 260). To date, most studies have used PCR in a qualitative way, that is, to obtain simple positive or negative results. If appropriate control groups are also studied, such results can provide a basis for studying the role of the virus in a given disease more rigorously but provide little immediate insight into pathogenic mechanisms.

Detection of cell-free virus in serum or plasma by PCR offers the possibility of diagnosing active HHV-6 infections. In such assays, neither HHV-6 variant was detected in a total of 57 healthy adults but viral DNA was detected in 16 (94%) of 17 ES patients, 3 (23%) of 13 BMT recipients, and 4 (22%) of 18 HIV-infected patients (227, 458). The timing of sample collection is a critical variable; only 3 (30%) of 10 ES patients were positive during the acute phase, while all 10 specimens collected from 7 to 20 days after the acute specimen were positive (227).

Quantitative methods for HHV-6 PCR have been developed (105, 112, 457) and are being further refined, as are methods for amplification and quantification of specific transcripts. A combination of quantitative variant-specific genomic PCR with quantitative detection of specific transcripts of various kinetic classes will be required to untangle many of the questions stimulated by observations that a disease-associated tissue is positive for HHV-6 DNA in a standard PCR assay.

Serodiagnosis

Standard immunoassay formats have been adapted for the detection of HHV-6 antibodies. These include indirect IFA (58, 64, 117, 292, 432), anti-complement immunofluorescence (58, 324), competitive radioimmune assay (118), neutralization (25, 486), and 96-well-based enzyme immunoassays (33, 59, 102, 120, 241, 400, 453). Several points should be emphasized about the current generation of assays. (i) The assays cannot easily discriminate infection with HHV-6A from infection with HHV-6B. In one report, fourfold or greater differences in IFA titers on HHV-6A and HHV-6B antigens were seen in only 15% of specimens (89). More convenient variant-specific serologic assays are being developed. (ii) HHV-6 and HHV-7 are sufficiently cross-reactive for false-positive results to be a concern unless serologic assays and antigen absorptions are performed for both viruses (59). (iii) Nearly everyone over the age of 2 years is seropositive for HHV-6, and thus a single positive result cannot be meaningfully interpreted.

IgM responses are frequently interpreted as being indicative of primary infection or a reactivation event. During primary infection, HHV-6 IgM antibodies can be detected within 5 to 7 days, but many culture-positive children do not develop detectable IgM responses (484). In addition, approximately 5% of healthy adults are IgM positive at any given time (484). CFS patients are more likely than controls to be positive for IgM antibodies to the HHV-6 p41/38 antigen, but a diagnostically useful cutoff has not been defined (402). Thus, IgM antibodies to HHV-6 do not provide a stand-alone diagnostic answer for any defined clinical entity.

Clinical Diagnosis

Given the limitations of the various assays when applied to individuals, how might such laboratory tests be used clinically for diagnosis and treatment of specific patients? Our thoughts on this question are as follows.

Febrile and rash illness. Virus culture from PBMC, PCR of PBMC, and assays of paired serum specimens (collected at least 1 week apart) provide the most direct evidence for HHV-6 activity, along with exclusion of other reasonable possibilities. For patients with classic ES, an extensive laboratory investigation is seldom necessary or economically justified. Should a rapid PCR or antigenemia test become available for routine clinical use, it could be useful in distinguishing cases of primary HHV-6 infection without typical ES manifestations from other illnesses with fever or exanthems and potentially prevent the inappropriate use of antibiotics. This latter point is important in an era of rapidly expanding antibiotic resistance among many pathogenic microorganisms.

Acute neurologic disease. PCR of CSF has been used to diagnose HHV-6-associated acute neurologic diseases (26, 289, 483, 565). A clinically available PCR of CSF could be beneficial for diagnosing HHV-6-related febrile seizures. Since

about one-quarter to one-third of first febrile seizures are associated with primary HHV-6 infection (47, 208, 529), such data could be reassuring by eliminating more serious diseases from the differential diagnosis. Confirmation could come from acute- and convalescent-phase serologic tests or from the finding in the acute-phase sample of low-affinity HHV-6 antibodies, suggestive of primary infection (532). A CSF PCR diagnostic test could also prove useful in testing patients with recurrent HHV-6-associated febrile seizures, as described by Kondo et al. (289), since such patients might be candidates for antiviral therapy. This consideration would not be likely to apply to most first-time febrile seizures, because complete resolution without long-term treatment is the rule for most children experiencing fever-related convulsions. Whether this is the case with the HHV-6-associated subgroup is in need of further study. Other patients who might benefit from a readily available HHV-6-specific diagnostic technique are those who experience an illness suggestive of viral encephalitis. Suspected HSV encephalitis is often treated with intravenous acyclovir on the basis of clinical and radiologic grounds, without invasive diagnostics. Immunocompromised hosts and others with findings or clinical courses atypical for HSV encephalitis would be more likely to undergo open brain biopsy for diagnosis. The finding of HHV-6-specific antigens by in situ IHC staining of biopsy specimens could indicate an alternative diagnosis and suggest the use of antiviral agents with anti-HHV-6 activity. PCR positivity of CSF for HHV-6 DNA may not be adequate for the diagnosis of HHV-6-associated encephalitis in these patients, as shown by the persistence of positive results after primary infection (84). Given the presently small database for the clinical conditions described here, this discussion represents points for consideration rather than firm recommendations. As more information about acute HHV-6-associated neurologic illness is collected, more refined guidelines can be formulated.

Other. For other suspected involvements of HHV-6 in disease, we suggest that a combination of virus culture, serum or plasma PCR, antigen capture EIA, PCR of the affected tissue, in situ IHC, and serologic tests (IgM and IgG on paired serum specimens) be used in conjunction with rigorous efforts to exclude other possibilities. In general, definitive diagnosis of HHV-6 involvement in diseases other than ES awaits further assay refinement and development as well as expansion of the database that will allow their interpretation.

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