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Laboratory and Clinical Aspects of Human Herpesvirus 6 Infections

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SUMMARY

Human herpesvirus 6 (HHV-6) is a widespread betaherpesvirus which is genetically related to human cytomegalovirus (HCMV) and now encompasses two different species: HHV-6A and HHV-6B. HHV-6 exhibits a wide cell tropism *in vivo* and, like other herpesviruses, induces a lifelong latent infection in humans. As a noticeable difference with respect to other human herpesviruses, genomic HHV-6 DNA is covalently integrated into the subtelomeric region of cell chromosomes (ciHHV-6) in about 1% of the general population. Although it is infrequent, this may be a confounding factor for the diagnosis of active viral infection. The diagnosis of HHV-6 infection is performed by both serologic and direct methods. The most prominent technique is the quantification of viral DNA in blood, other body fluids, and organs by means of real-time PCR. Many active HHV-6 infections, corresponding to primary infections, reactivations, or exogenous reinfections, are asymptomatic. However, the virus may be the cause of serious diseases, particularly in immunocompromised individuals. As emblematic examples of HHV-6 pathogenicity, exanthema subitum, a benign disease of infancy, is associated with primary infection, whereas further virus reactivations can induce severe encephalitis cases, particularly in hematopoietic stem cell transplant recipients. Generally speaking, the formal demonstra-

Published 11 March 2015

Citation Agut H, Bonnafous P, Gautheret-Dejean A. 11 March 2015. Laboratory and clinical aspects of human herpesvirus 6 infections. Clin Microbiol Rev doi:10.1128/CMR.00122-14.

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doi:10.1128/CMR.00122-14

tion of the causative role of HHV-6 in many acute and chronic human diseases is difficult due to the ubiquitous nature of the virus, chronicity of infection, existence of two distinct species, and limitations of current investigational tools. The antiviral compounds ganciclovir, foscarnet, and cidofovir are effective against active HHV-6 infections, but the indications for treatment, as well as the conditions of drug administration, are not formally approved to date. There are still numerous pending questions about HHV-6 which should stimulate future research works on the pathophysiology, diagnosis, and therapy of this remarkable human virus.

INTRODUCTION

In 1986, the discovery of a novel human herpesvirus was reported in the journal *Science* (1). The importance of the finding was amplified by the fact that this virus, finally designated human herpesvirus 6 (HHV-6), was initially isolated from patients with lymphoproliferative disorders and AIDS, raising the key question of its pathogenicity. Since that time, the knowledge about this virus and associated diseases has considerably improved, as extensively reported in previously published overviews and books which remain relevant references (2–10). However, many questions on those domains are still pending and constitute true challenges for present and future research. How current tools of medical virology and clinical studies may help researchers to investigate the pathophysiology of HHV-6 infection and consider the means of its control constitutes the core subject of the present review.

(Some of the data and concepts reviewed in the present text have been discussed previously in part in an article referenced herein [11] and in oral communications at the 7th and 8th International Conferences on HHV-6 and HHV-7, which took place in Reston, VA, in 2011 and in Paris, France, in 2013, respectively.)

VIRUS PROPERTIES

Discovery and Classification

HHV-6 was first isolated from the peripheral blood mononuclear cells of patients with lymphoproliferative disorders in attempts to characterize novel lymphotropic human viruses (1). A cytopathic effect made of short-lived large refractile cells was observed in the primary cell cultures and was shown to be transmissible to novel cultures of phytohemagglutinin (PHA)-stimulated human leukocytes. Electron microscopy confirmed virus production and revealed a morphology of virus particles similar to that of herpesviruses. This included a capsid of icosahedral symmetry surrounded by a tegument within an enveloped particle of about 200 nm in diameter (12). The genomic DNA did not cross-hybridize with the genomes of the five other known human herpesviruses, unambiguously demonstrating that the newly isolated virus was different from them (13). HHV-6 was initially characterized as a human B-lymphotropic virus, but it soon appeared that it was essentially a T-lymphotropic virus, and it acquired its definite name (14). Finally, based on both biological properties and genetic analyses, HHV-6 was officially classified as a member of the *Herpesvirales* order, *Herpesviridae* family, *Betaherpesvirinae* subfamily (the type species of which is human cytomegalovirus [HMCV]), and *Roseolovirus* genus, together with human herpesvirus 7 (HHV-7), a closely related herpesvirus discovered in 1990 (15). Following the description of the initial HHV-6 strain, named GS, other prototypic HHV-6 strains, designated U1102, SIE, LHV, Z29, and HST, were obtained in other laboratories, from HIV-infected patients, mostly of African origin, but also from Japanese patients with exanthema subitum (16–20). Those isolates and the HHV-6 isolates obtained subsequently were stratified into two well-defined, nonoverlapping groups differing by specific genetic changes and phenotypic properties. These two groups were designated variants A and B (HHV-6A and HHV-6B) of the unique HHV-6 species (21). Twenty years later, the differences between the two variants have been considered important enough to recommend the classification of HHV-6A and HHV-6B as two distinct species (22). The term HHV-6 remains in usage and collectively refers to the two species.

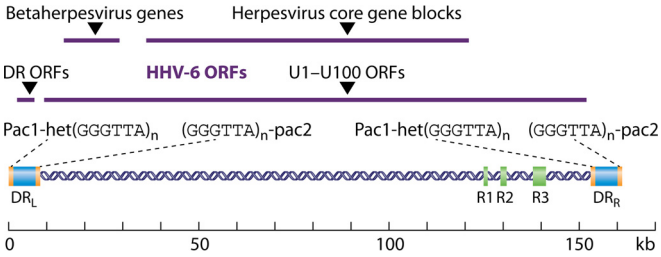


FIG 1 Schematic representation of the HHV-6 genome. The genome is represented as a double-stranded DNA containing specific elements which are described in the text. The repeat elements, shown as green and blue boxes, include the identical terminal repeat sequences DR_L and DR_R, the internal repeat arrays R1, R2, and R3, and the stretches containing repeat hexanucleotide sequences [$(GGGTTA)_n$] within the DR regions. Purple straight lines indicate the positions of the different sets of ORFs.

Genome and Genetic Variability

The genome of HHV-6 is a linear double-stranded DNA consisting of a unique (U), 143- to 145-kb region flanked by identical terminal direct repeats (DR_L and DR_R) and having an overall approximate length of 162 to 170 kb (Fig. 1). The U region also contains internal repeat arrays designated R1, R2, and R3. DR_L and DR_R each contain short unique sequences, the conserved cleavage-packaging motifs pac-1 and pac-2, and two stretches of sequences related to the telomere repeat sequences (TRS) of vertebrate chromosomes (5). The one near the left end of each DR contains reiterations of the hexanucleotide GGGTTA interspersed by related but different sequences, constituting the heterogeneous telomeric-like region het$(GGGTTA)_n$. The right end of each DR contains perfect repeats of GGGTTA. These repeated sequences likely play a major role in the process of chromosomal integration of the HHV-6 genome (ciHHV-6) (see below). This integration has been reported to occur through a junction between host chromosomes and the perfect telomeric repeats at the right end of DR_R (23–25). The overall number of protein-encoding open reading frames (ORFs) is about 110 to 120 according to the different published nucleotide sequences (26–28). Most of them are located within the U region, on both genomic strands. This unique region presents strong similarities with that of HHV-7 DNA and the unique long region of the HCMV genome. The core genes coding for the virion proteins and enzymes involved in the virus replication cycle regroup into seven clusters, with each block being composed of two to eight ORFs which are conserved among all herpesviruses and overlay the central part of the U region. In addition, a betaherpesvirus-specific gene cluster and a roseolovirus-specific set of genes are present at the left end of the core genes. Several genes, such as U83 and U94, are unique to HHV-6.

The genetic variability of HHV-6 DNA is limited, with the nucleotide identity among all published sequences exceeding 90% as a whole. However, this identity rate differs according to the genes considered, ranging from about 70% for the right part of the genome to about 95% for the central core genes. This genetic polymorphism can be analyzed at three different levels of complexity. As mentioned above, HHV-6A and HHV-6B DNAs exhibit clear specific differences which are scattered throughout the genome and permit their easy recognition, without any ambiguity. These specific signatures concern the rather variable genes of immediate early region 1 (IE1) as well as the highly conserved genes for glycoproteins B (gB) and H (gH) and the U94 product (29–31). Some of the HHV-6A ORFs are thought to have no HHV-6B counterpart and *vice versa*, although the prediction of functional ORFs often remains debatable in the absence of relevant experimental investigations. Taken as a whole, the differences between HHV-6A and HHV-6B are believed to induce noticeable dissimilarities in the virus replication cycle, in particular regarding splicing patterns and the temporal regulation of gene transcription. Those differences may also affect the binding of viral proteins to their cellular targets, which in turn may alter cell tropism, interactions with the microenvironment, host immune responses, and, ultimately, pathogenesis (4, 22). Note that no recombinant virus originating from a mixed infection with HHV-6A and HHV-6B has ever been identified, although recent results provide some evidence of recombination between ciHHV-6A and ciHHV-6B (32). At an intermediate level of variability, the segregation of strains into distinct subgroups characterized by specific genetic signatures has been reported for HHV-6B isolates but not HHV-6A ones, based on the analysis of IE1, gB, and gH genes (29, 33). However, this segregation is not fully congruent, since it may differ according to the selected gene: the subgroups derived from the phylogenetic analysis of gB gene sequences did not exactly fit those derived from gH gene sequences. Conversely, the study of gB gene polymorphisms provided indirect evidence for genetic recombination among HHV-6B subgroups, suggesting that this genetic process may contribute to creating novel allelic combinations among these putative subgroups. The question remains as to whether the segregation into subgroups is not simply a misinterpretation of basic interstrain polymorphism, which constitutes the lowest level of variability. Overall, this genetic variability is low in terms of nucleotide sequence, and even lower in terms of amino acid sequence (34). However, despite its modest magnitude, the interstrain variability may provide useful markers for differentiating viruses in molecular epidemiology studies (35). A particular aspect concerns the number of TRS in the DR region, which does not depend on the classification as HHV-6A or HHV-6B: it varies widely among the different strains studied but remains stable for a given isolate, even after numerous serial passages in cell culture, and that property can be used to track specific HHV-6 strains in human infections (36, 37). Nevertheless, previous reports mentioned that the length of the DR region changed on viral passage in cell culture in the case of HHV-6B strain Z29 (27, 38).

Cell, Tissue, and Host Tropism

HHV-6 infects a wide range of human cells *in vitro*, but it preferentially replicates in activated $CD4^+$ T lymphocytes (3, 5). At least one component of the cell receptor permitting virus anchorage to the cell surface differs according to HHV-6 species: HHV-6A uses CD46, a regulator of complement activation expressed on all nucleated cells, while CD134 (also called OX40), a member of the tumor necrosis factor (TNF) receptor superfamily present only on activated T lymphocytes, functions as a specific entry receptor for HHV-6B (39, 40). Note that CD46 is a receptor for other human pathogens, including the vaccine strains of measles virus and *Neisseria gonorrhoeae*, behaving as a pathogen magnet (41). In addition to $CD4^+$ T lymphocytes, HHV-6 can infect *in vitro* $CD8^+$ T lymphocytes (only with HHV-6A), human fibroblasts, natural killer cells, liver cells, epithelial cells, endothelial cells, astrocytes, oligodendrocytes, and microglial cells. However, its capacity to infect continuous T cell lines is limited, and in many cases, it can be obtained only through an adaptation process consisting of serial blind passages of a primary isolate on the target cells. The capability to infect different cell lines is generally higher for HHV-6A than for HHV-6B and appears to be a phenotypic character for discriminating both species. As a whole, no continuous cell line can be recommended for isolation of the virus. The primary isolation of HHV-6 from a human specimen usually requires cocultivation with primary highly susceptible cells consisting of peripheral blood mononuclear cells (PBMCs) or umbilical cord blood lymphocytes.

As for HCMV, the host tissue range of HHV-6 *in vivo* appears to be broader than might be expected from *in vitro* studies and includes the brain, tonsils, salivary glands, kidneys, liver, lymph nodes, endothelial cells, and monocytes/macrophages (3, 42–48). The latter cell types are suspected to be preferential sites for virus latency, in parallel with bone marrow progenitors and central nervous system (CNS) cells (49–51). Although HHV-6 infection is naturally restricted to human cells and tissues, simian cells and monkeys can be infected experimentally, but the availability of this model is extremely low (52, 53). In transgenic mice expressing human CD46 and infected with HHV-6A, the virus persisted in the brain for months, and a significant inflammatory response developed, opening the possibility of a rodent model for virus-induced neuroinflammatory diseases (54, 55).

Replication Cycle

HHV-6 attaches to its cell receptor by means of a tetrameric viral ligand complex made up of the glycoproteins H (gH), L (gL), Q1 (gQ1), and Q2 (gQ2) (8). Following attachment, HHV-6 entry into cell occurs through a fusion between the viral envelope and the cell membrane by a mechanism which involves gB and gH functions but remains poorly understood. The nucleocapsid is then transported through the cytoplasm to the nucleus, likely using the pathway of the microtubule network. HHV-6 DNA is released into the nucleoplasm. Viral genes are expressed in a temporally ordered manner, starting with immediate early (IE) genes from the IE-A locus, which is constituted of two genetic units, IE1 and IE2 (56–58). Those genes are transcribed in the absence of *de novo* protein synthesis, and this step is followed by the transcription/expression of early (E) and late (L) genes. The replication of the genome occurs after the synthesis of E proteins, which have enzymatic activities dedicated to nucleotide metabolism and DNA synthesis, i.e., phosphotransferase, ribonucleotide reductase, uracil-DNA glycosylase, origin-binding protein, DNA polymerase, polymerase processivity factor, major DNA-binding protein, and helicase-primase complex activities. Viral DNA is assumed to be replicated through a rolling circle process. Progeny DNA is yielded in the form of concatemeric strands, which are cleaved and pack-

aged into capsid precursors thanks to specific cleavage-packaging signals present in the DR_L and DR_R regions (8). The capsids exit the nucleus, acquiring an intermediate envelope by budding through the inner part of the nuclear membrane, are deenveloped by fusion with the external part of this membrane, and appear as tegumentary forms in the cytoplasm. The acquisition of the final envelope carrying viral glycoproteins occurs in the *trans*-Golgi network, and mature virions are released by exocytosis. The occurrence of a complete replication cycle, which lasts about 3 days, has a major impact on host cell functions and morphology. Infected cells engaged in this virus-producing process ultimately die by apoptosis and/or necrosis.

Latency and Reactivation

Like other human herpesviruses, HHV-6 persists indefinitely in its host and is capable of reactivation, meaning the active production of detectable mature virions in some body compartments following a phase of apparently complete clearance. These properties rely on the putative capacity of its genome to be maintained in a nuclear latent form or to drive a low-level productive infection in some cells while inducing a fully lytic infection in other cells. For other human herpesviruses, such as herpes simplex virus, the latent DNA genome has the form of a covalently closed circular episome associated with cellular nuclear proteins. The existence of such a latent nuclear form has not been demonstrated formally for HHV-6, although an episomal state was shown after experimental infection of cervical carcinoma cell lines (59).The viral gene U94, which is expressed during latent infection, is assumed to play a major role in the establishment and maintenance of intracellular latency (60). Other latency-associated transcripts have also been described (61). Reactivation occurs through the transcription of IE genes in the IE1 and IE2 regions following the likely transactivation effect of cellular and/or viral factors whose nature is still unknown. This reactivation process results in the induction of a replication cycle and the possible appearance of a cytopathic effect (48).

Chromosomal Integration

The integration of the HHV-6 genome into human chromosomes (ciHHV-6) was initially described for transformed cell lines (62, 63). This phenomenon was further reported to be present in human cells *in vivo*, including cells which can be transmitted as germinal cells to offspring and hematopoietic stem cells transferred to a transplant recipient (64, 65). It appears to be a unique feature among human herpesviruses and raises numerous novel questions regarding both pathophysiology and diagnosis (25, 66). The covalent linkage between viral and cellular DNAs occurs within the subtelomeric region of chromosomes, likely by a mechanism of homologous recombination between telomeric repeat sequences of viral and cellular origins. The phenomenon has been described for both HHV-6A and HHV-6B and occurs in 0.2 to 1% of the general population in developed countries. It might be generated in the context of *de novo* infection and is considered by some authors to be the default pathway of HHV-6 latency, including in non-germ line cells and before persistence of viral DNA as an episome (23). Although there is no *in vivo* evidence for that assumption, it must be kept in mind knowing that HHV-6 has the ability to infect sperm cells (67, 68). Thus, *de novo* HHV-6 infection of germinal cells might result in individuals harboring the integrated virus in their germ line and transmitting it to their offspring (25). Moreover, ciHHV-6 might lead directly to reactivation, as reflected by the production of viral transcripts, proteins, and even transmissible virions (23, 35, 69). This emphasizes the tight relationship between ciHHV-6, latency, and reactivation. In that context, it is worth recalling the homology between HHV-6 U94 and the human adeno-associated virus (AAV) type 2 *rep* gene (70). *rep* gene products are involved in the site-specific integration of AAV DNA into host cells. Therefore, U94 products might have a pivotal role both in the establishment of latency and in ciHHV-6.

Impacts of Viral Gene Expression on Cell Functions

As previously mentioned, the occurrence of a complete replication cycle has profound effects on cell functions and viability in the context of either *de novo* infection or reactivation. In addition, independently of any complete virus-producing process, the expression of certain HHV-6 genes might occur from persisting episomal or ciHHV-6 forms of viral DNA. Many publications have reviewed the formally demonstrated or putative effects of virally encoded gene products on the regulation and modification of cell functions (3, 4, 8). As an example, considering the gene products of the IE-A region, IE2 might behave as a general transcriptional activator of many viral and cellular genes, while IE1 interacts with PML bodies (71, 72). The proteins encoded by the IE-B region have also been shown to transactivate heterologous promoters, such as the HIV-1 long terminal repeat (LTR) (30). The products of the DR7 gene appear to demonstrate a cell-transforming activity, presumably through an interaction with p53 (73). Regarding the U94 gene, which is analogous to the AAV *rep* gene, it can bind to the human TATA-binding protein, and its expression in endothelial cells decreases cell migration and angiogenesis (74, 75). The U95 gene product interacts with the mitochondrial GRIM-19 protein, a component of the oxidative phosphorylation system involved in apoptotic processes (76). As indicated below, several proteins encoded by the HHV-6 genome have immunomodulatory functions. Taken together, all these features provide molecular bases for understanding the pathological processes associated with acute and chronic HHV-6 infections.

HUMAN INFECTION

Epidemiology

HHV-6A and HHV-6B are ubiquitous viruses that are detected in all human populations around the world, as reviewed elsewhere (3, 5, 8). Current serologic assays do not permit discrimination of HHV-6A and HHV-6B infections (77). Consequently, a precise view of their respective seroprevalences in different human populations is not available at present. As a whole, HHV-6 infection is detected in more than 90% of adult populations in developed countries, although the data on seroprevalence may reveal significant differences according to geographic location, age of subjects, and sensitivity and specificity of serologic assays. Standardized species-specific serologic tests will help to clarify the meaning of these differences and to determine whether the circulation of HHV-6A and HHV-6B truly differs in different ethnic groups, countries, or continents.

HHV-6 infection is usually acquired very early in life, between 6 months and 2 years of age, following the loss of protective maternal antibodies (78). At an even earlier period of life, congenital infection following intrauterine transmission has been reported for about 1% of children, a frequency close to that observed with

HCMV, and cases of perinatal transmission have been described (79–82). As described below, congenital infection is mainly linked with ciHHV-6 in mothers (78). Primary infection can also happen later, in adults, as reported in a few cases (83). Saliva is assumed to be the main vehicle for virus transmission, as supported by the frequent detection of HHV-6 in saliva and salivary glands. Virus transmission through organ transplantation has been described infrequently, while blood transfusion and breast feeding have never been reported to be origins of primary infections (84, 85). To date, a clear view of the respective temporality of HHV-6A and HHV-6B infections is missing. It is generally believed that in the majority of countries, primary HHV-6B infection occurs first, in many cases associated with clinical symptoms, whereas HHV-6A is acquired later, through asymptomatic infection (see below). Ultimately, the concomitant detection of HHV-6A and HHV-6B in blood or tissues from adults indicates that the two viruses chronically infect many, if not most, individuals (86, 87).

Physiology

No experimental *in vivo* model of human infection is currently available. Several monkey species are infected with HHV-6-related viruses, but their capability to provide a relevant model regarding HHV-6 pathophysiology is still unknown (88, 89). Monkeys as well as transgenic mice expressing human CD46 could be infected with HHV-6A and developed a specific antiviral immune response; in some cases, neurologic symptoms and neuropathological lesions were observed (54, 55, 90, 91). However, to date, none of these animal models has given a conveniently workable picture of the course of human infection. This course can only be hypothesized from clinical and biological findings observed during the natural process (92). Clinical symptoms as well as virological data reported for primary infection cases suggest that following its entry into the body by the oral route, the virus replicates in the salivary glands and satellite lymphoid tissues of the oropharynx, probably the tonsils and cervical lymph nodes. Systemic diffusion of infection would occur by the blood route, taking advantage of the virus tropism for PBMCs and vascular endothelial cells, as reflected by the isolation of infectious virus from blood during the acute phase of infection. A spreading of virus and infected cells via lymphatic vessels is also possible. This spreading leads to the active, abortive, or latent infection of susceptible cells in other organs, including T lymphocytes and monocytic cells in lymphoid tissue and the liver, kidney and skin epithelial cells, hematopoietic stem cells in bone marrow, and neuroglial cells in the CNS. The entry of HHV-6 into the CNS might occur by crossing of the blood-brain barrier through the olfactory pathway (93). In most cases, this primary infection is self-limiting while a specific immune response develops (see below), and the viremia finally decreases to undetectable levels as measured by conventional diagnostic assays.

The establishment of HHV-6 latency in the body raises numerous questions regarding chronology, cell location, gene expression level, and potential differences between HHV-6A and HHV-6B. HHV-6 DNA is detected, in particular, in saliva, blood monocytes, endothelial cells, and bone marrow progenitors (48, 50, 94). As mentioned previously, latency-associated transcripts from U94 and the IE region have been detected in PBMCs (8). Currently, it is not known whether the transcription of other genes, synthesis of proteins, and even production of virions can occur at low levels in particular cells, tissues, or organs during the latency stage of infection, which would make the putative frontier between latent and active infections even narrower (95). Some authors have suggested that HHV-6A does not exhibit the same capability to establish latency in target cells as that of HHV-6B. However, this hypothesis has to be modulated by the fact that regardless of infection stage, and for still unknown reasons, HHV-6B is detected much more frequently than HHV-6A in PBMCs and cerebrospinal fluid (CSF) (8). As mentioned above, ciHHV-6, present in about 1% of the adult population, is another form of virus quiescence in which each cell in the body contains one copy of the viral genome.

Reactivation can be defined as the reappearance of replication cycle transcripts and yield of infectious virus in peripheral blood or in a specific tissue or organ from an individual who has experienced a primary infection. This may lead to extended reinfection of bodily tissues and cause disease. The initial cellular events can be induced experimentally in cultures of latently infected cells following exposure to a phorbol ester, such as tetradecanoylphorbol-13-acetate (TPA), or superinfection with HHV-7 (48, 96). Note that stimulation using TPA or trichostatin A has been shown to promote virus activation from ciHHV-6 in PBMCs as well as in cell lines (23). *In vivo*, the recognition of reactivation may reveal more complexity due to heterogeneity of patient presentations and limitations of virological methods used to address this issue (see below). In particular, endogenous reactivation has to be distinguished from reinfection of exogenous origin with a novel strain of HHV-6 (11). Exogenous reinfection in an individual who is already seropositive with respect to the same virus species, either HHV-6A or HHV-6B, is assumed to occur, as reflected by the concomitant detection of distinct viral strains by molecular epidemiology approaches (36). However, the frequency of that phenomenon, as well as its pathophysiological consequences, is still unknown.

Interactions with the Immune System

A specific immune response to HHV-6 was recognized very soon after its discovery (8, 97–99). For patients experiencing primary infection, serologic studies have shown the appearance of specific IgM antibodies during the first week and their subsequent disappearance after 1 month, while IgG antibodies are detected later than IgM but persist indefinitely. These antibodies react with a wide range of virally encoded proteins, some of which are considered major antigens by immunoblot studies, such as the U11 gene product. Some of these antibodies have a virus-neutralizing activity, but their activity in the control of active infection is not well understood. Cellular immunity is believed to play the major role in this control, as reflected by the deleterious effects of T cell immune response suppression. Recent publications reported the proliferation of $CD4^+$ and $CD8^+$ T cells in response to HHV-6A and HHV-6B antigens in most healthy adults (100–102). Those studies permitted the fine characterization of a significant number of HHV-6 T cell epitopes but showed the following two important limitations for future investigations: the low frequency of circulating HHV-6-specific T cells, requiring *in vitro* expansion prior to any functional characterization; and the high degree of cross-reactivity between HHV-6A and HHV-6B epitopes. Prior to the emergence of adaptive immunity, HHV-6 infection has the capacity to stimulate the effectors of innate immunity: an increased secretion of proinflammatory cytokines, such as interleukin-1β (IL-1β), TNF-α, and alpha interferon (IFN-α), is observed in

PBMCs, while NK cell activity associated with IL-15 synthesis is elevated in HHV-6A infection (103, 104).

Like other herpesviruses, HHV-6 exhibits a wide range of biological properties which might explain its ability to both stimulate and modulate immune responses (3, 4, 8). This modulation, in turn, would permit evasion of the HHV-6-specific immune response and improve the microenvironmental conditions for promoting virus persistence. As an example, the upregulation of proinflammatory cytokines in PBMCs is associated with a downregulation of IL-2 synthesis and a subsequent decrease of T cell activation. Accordingly, HHV-6 has been shown to promote the shift of the T-helper cell profile from Th1 to Th2 by upregulating IL-10 and downregulating IL-12. HHV-6A infection has been shown to downregulate the expression of HLA class I expression on dendritic cells. In parallel, HHV-6 infection has strong suppressor effects on the growth and differentiation of bone marrow progenitors, which may affect the differentiation of macrophages and the population of thymocyte precursors. Many of these effects are specifically mediated by HHV-6-encoded proteins which act as analogues of cell chemokines and are believed to promote viral growth, viral dissemination, and/or escape from the immune response. The U21 protein has been shown to reduce the expression of HLA class I expression as mentioned above. As other examples, the U83 gene encodes a chemotactic protein which is an agonist for several human CC chemokine receptors (CCRs) and the U12 and U51 genes encode chemokine receptors which presumably activate and recruit host cells (105, 106). The U24 gene product induces the internalization of the T cell receptor/CD3 complex, which may alter the patterns of T cell activation. *In vitro* studies have often provided conflicting evidence concerning the effects of these proteins, depending on the type of cells and the HHV-6 species investigated. However, it seems clear that the virus has the capacity to perform a fine-tuning regulation of cell functions. This capacity is likely extended to the modulation of other viral infections affecting the same target cells and organs. The role of HHV-6 as a cofactor of HIV in AIDS remains a matter of discussion. It is substantiated by several *in vitro* findings relying on interactions between both viruses, i.e., the common tropism of HHV-6 and HIV for $CD4^+$ T cells, transactivation of the HIV-1 LTR by HHV-6 proteins, and induction of CD4 expression on $CD8^+$ T cells and NK cells by HHV-6 making these cells susceptible to HIV infection (107). HHV-6 infection has also been shown to stimulate the activation of Epstein-Barr virus (EBV) from latency and, more recently, the expression of the human endogenous retrovirus K-18 (108, 109).

VIROLOGICAL DIAGNOSIS

Objectives and Means

The aim of virological diagnosis is first to provide proof of HHV-6A or HHV-6B infection, i.e., the presence of the virus(es) in an investigated subject. Second, it is necessary to define the status of this infection as latent, active (which can also be termed productive or acute), or ciHHV-6 related. Third, the viral load and, if possible, the expression of viral genes have to be quantified in peripheral blood and specific body compartments in order to determine the possible causative relationship with the concomitant clinical symptoms. Fourth, the question of treating the infection with antiviral drugs active against HHV-6 has to be considered. Consequently, diagnostic procedures are implemented for the monitoring of infection following initial diagnosis and therapy, if started. This includes the serial quantification of virus replication and the detection of putative resistance to antivirals in case of therapeutic failure (110).

Additional objectives refer to the study of interhuman transmission cases and the phylogenic relationships between viral strains and viral subpopulations in the context of a mixed infection within the same individual. These require the implementation of molecular studies based on gene amplification and nucleotide sequencing. Since HHV-6A and HHV-6B are now defined as distinct viral species, it is essential that differentiation between them be obtained at an early step of any HHV-6 infection diagnostic procedure, and this is also required for publication of HHV-6-related scientific articles (22).

Diagnostic procedures performed for either the management of a sole patient or the planned study of a human cohort are basically similar (Table 1). A wide range of human specimens can support these procedures, with the most common ones being whole blood, plasma, and serum. Cerebrospinal fluid is essential for the diagnosis of central nervous system infections, while bronchoalveolar lavage permits investigation of lung infections. The relevance of saliva samples for diagnosis needs to be clarified, as is the case for any cell fraction or extract obtained from a body fluid, cell smear, or tissue biopsy specimen (111). However, the frequent detection of HHV-6 in saliva and the capacity of saliva to contaminate lower respiratory tract specimens have to be taken into account in the interpretation of results.

Two general complementary approaches can be used (110). Direct diagnosis is based on the detection and characterization of whole virions or some of their specific components, the most convenient of which currently are nucleic acids. These components may come from either cell-free virions or infected cells. Infected cells contain not only the components of released viral particles but also transcripts and additional virus-encoded proteins, which provides an even larger set of viral targets. The indirect approach, also known as serology, is based on the detection and characterization of virus-specific antibodies in a body fluid, usually serum, using reference viral antigens. Due to the stability of antibodies even after prolonged storage of samples, serologic assays provide reproducible results and are very convenient for retrospective studies. However, the interpretation of serologic results may be equivocal for many reasons that can be summarized as follows. HHV-6A and HHV-6B infections are widespread and lifelong in the general population, making seropositivity highly prevalent and poorly discriminant. Rapid increases in IgG responses and the presence of IgM antibodies are not highly specific for acute primary infections, since these phenomena may also be associated with virus reactivations. The serologic profile may be atypical in the case of immune suppression or ciHHV-6. Cross-reactivity has been reported between antibodies to the four human betaherpesviruses, i.e., HHV-6A, HHV-6B, HHV-7, and HCMV. Lastly, commercially available serologic assays targeting HHV-6 antibodies cannot differentiate HHV-6A from HHV-6B infections to date.

Serology

Serologic methods are mainly founded on indirect immunofluorescence assays (IFA). In such assays, HHV-6-infected cells are fixed on glass slides, incubated with serum, and observed with an optical microscope. The readout of the reaction relies on both the

TABLE 1 Overview of diagnostic procedures for HHV-6 infection

Diagnostic approach	Method	Advantages and usefulness	Disadvantages and limitations[b]
Indirect (serology)	Assays for IgG and IgM detection (IFA, ELISA)[a] and avidity assays	Easy collection and storage of serum samples, readily accessible techniques, diagnosis of primary infection, seroprevalence studies	Lack of interpretation for diagnosis of reactivations, no discrimination between HHV-6A and HHV-B, delayed/altered response if immune deficiency is present, cross-reactivity with other betaherpesviruses
Direct	Virus isolation in cell culture	Reference method in virology, evidence of infectious virus, precise investigations of virus strains	Labor-intensive method, high cost, limited sensitivity
	Antigen detection	Uses conventional equipment, gives evidence of virus gene expression, discrimination between HHV-6A and HHV-6B	Need for standardization, limited sensitivity with current reagents, difficulties of readout in some cases
	Qualitative viral DNA PCR	High sensitivity and specificity, discrimination between HHV-6A and HHV-6B	No distinction between active infection, latency, and ciHHV-6
	Quantitative viral DNA real-time PCR	High sensitivity and specificity, discrimination between HHV-6A and HHV-6B, longitudinal follow-up studies, comparison of viral loads in blood versus organs	Need for international standardization, need for specific thresholds for active infections and ciHHV-6
	Detection of viral transcripts by RT-PCR	Distinction between active and latent infections, recognition of active infection in ciHHV-6 subjects	Limited sensitivity (to be evaluated), need for standardization
	Droplet digital PCR	Precise method for measuring nucleic acid amounts, identification of ciHHV-6	Limited sensitivity (to be evaluated), adaptation to clinical specimen diversity

[a] IFA, immunofluorescence assay; ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcriptase PCR; ciHHV-6, chromosomally integrated human herpesvirus 6.
[b] The availability of the mentioned tests may be restricted in some medical centers.

number of fluorescent foci and characteristic patterns of cell staining, also taking into account the intensity of nonspecific background fluorescence signals. The search for neutralizing antibodies is motivated by their specificity and their putative correlation with protective immunity, but this technique is cumbersome and expensive (112). A few enzyme-linked immunosorbent assays (ELISAs) have been developed and commercialized, using either a crude lysate of infected cells or purified virus obtained from cell culture supernatant as the antigen (113), but the question of their specificity has been raised repeatedly. The use of synthetic peptides as antigens is expected to improve their quality in the future, as well as the extension of the use of immunoblot assays or measurements of antibody avidity (114, 115). However, it is not certain that this evolution can circumvent some of the shortcomings mentioned above, in particular the difficulty in interpreting the presence of IgM and the cross-reactivity sometimes observed between distinct betaherpesviruses (116). Clearly, a better knowledge of the humoral immune response against HHV-6 at different stages of infection (primary production, latency, and ciHHV-6) is required to permit significant advances in this domain. The main indications of serologic assays currently remain the diagnosis of primary infection, identification of HHV-6-naive subjects, and use for seroprevalence studies. Consequently, HHV-6 serology has limited usefulness in the management of adult infections (77).

Direct Diagnosis

The isolation of HHV-6A, HHV-6B, and HHV-7 in cell cultures is a reference method and unambiguously demonstrates the presence of infectious viral particles in a sample. However, this method is poorly sensitive, time-consuming, and expensive. It cannot be used for routine diagnosis and is not available in most centers. All HHV-6 isolates theoretically grow on PBMCs or cord blood lymphocytes, leading to a cytopathic effect made of enlarged and refractive cells, which might be missing in some cases. Isolation on other cell types, such as fibroblasts, and growth adaptation on cell lines are possible in some cases but even more difficult than culture on primary permissive cells.

The detection of HHV-6 antigens in PBMCs and tissue biopsy specimens enables observation of viral proteins expressed at different stages of infection and even provides an approximate quantification of this process (117, 118). This is particularly useful for showing active viral infection at the site of tissue lesions by use of immunohistochemistry techniques. However, the panel of available reference antibodies that can be used to develop such assays is limited, and the sensitivity of detection is considered low with current reagents. This explains why current antigen detection studies and histological investigations are performed mainly for research objectives rather than diagnostic procedures.

The detection, quantification, and sequencing of HHV-6 nucleic acids have been combined to become the gold standard of diagnostic procedures applied to HHV-6A and HHV-6B. By means of numerous assays based on real-time PCR, HHV-6 DNA can be detected and reproducibly quantified in a broad range of clinical specimens, including whole blood, cerebrospinal fluid, and any other bodily fluid or tissue (110, 119, 120). The methods are financially accessible, quick, safe, and currently widespread. Most of the previous problems related to the nonspecific inhibition of DNA amplification and carryover inside labs have now been solved. In addition, these approaches also readily permit differentiation of the two species of HHV-6, even in cases of mixed infection (121). However, there is an obvious need for standardization of the various molecular assays in use in order to permit the unambiguous comparison and interpretation of results obtained in different laboratories; in that context, the introduction of a reference international standard would con-

stitute a significant advance, as observed recently for HCMV diagnosis (122).

The detection and quantification of viral transcripts appear to comprise a valuable complementary approach, with the theoretical possibility of recognizing the different steps of the virus cycle. With that purpose, the detection of late gene transcripts would help to identify productive infections, whereas the finding of a remotely detectable amount of U94 transcripts would reveal a predominantly latent phase of infection. The detection of transcripts is facilitated by targeting of spliced mRNAs, with the amplimers obtained from transcripts thus being distinctly shorter than those amplified from genes containing introns. However, the knowledge of the different transcription patterns of HHV-6 is currently far from complete, and the methods of mRNA quantification are not yet properly standardized.

The need for a precise molecular characterization of HHV-6 DNA and transcripts has emerged from recent questions addressing the epidemiology and physiology of HHV-6 infections. These questions concern the classification of HHV-6A and HHV-6B into two different species, as well as the understanding of transplacentally acquired congenital infection and reactivation from the ciHHV-6 stage (29, 35, 69). The general strategy is the combination of gene amplification, nucleotide sequencing, and phylogenic study of selected loci of HHV-6 DNA. This also allows the genetic recognition of resistance to antivirals by targeting the search for specific mutations known to confer a decreased susceptibility to drugs (123, 124). The most recent developments of molecular techniques, namely, droplet digital PCR and next-generation sequencing, will offer novel opportunities for the accurate investigation of infection pathophysiology. In particular, these developments will permit us to address complex molecular phenomena, such as coinfections of HHV-6A and HHV-B, ciHHV-6, and modulation of gene expression (125–127). In addition, they might help to map chromosomal integration sites in ciHHV-6 subjects in parallel with the classical cytogenetic approach, which uses fluorescence *in situ* hybridization.

INTERPRETING VIROLOGICAL RESULTS

Active Infection

One goal of virological investigations is the distinction between active (or acute) and latent infections, although this distinction is made difficult by the absence of a neat frontier between the two stages of infection. In terms of pathophysiology, active infections correspond to primary infections, endogenous reactivations, and exogenous reinfections (11). They tend to attract a great deal of attention because they are more accessible to current direct diagnosis procedures, are potential targets for specific antiviral therapy, which so far is directed mainly against HHV-6 DNA replication, and can be correlated more convincingly with concomitant disease.

Detectable viremia is generally considered the hallmark of a systemic active infection (4). According to several authors, whole blood is the most valuable specimen for detecting such viremia by means of real-time PCR (120, 128). Alternatively, the use of PBMCs for such detection is justified, since HHV-6 remains mostly cell associated during active infection. In contrast, the use of plasma, albeit more convenient, raises controversy regarding the origin of viral DNA present in this blood compartment, corresponding to either a true virus production from lymphoid tissue or an incidental DNA release from the lysis of circulating cells. The high sensitivity of real-time PCR and ubiquitous presence of latent HHV-6 infection in adults lead to a high frequency of positive qualitative detection of viral DNA. Therefore, for a proper interpretation, the HHV-6 DNA load in blood has to be computed precisely. So far, no threshold has formally been defined as the frontier between latent and active infections. As a preliminary approximation, the threshold of 1,000 genome-equivalent copies per ml of whole blood delineates a fluctuating gray zone separating the two stages of HHV-6 infection (129–131). This broadly corresponds to 1,000 copies per million PBMCs when the white blood cell count is within the normal range. Viral loads corresponding to active infections are thus located within a wide range, from 1,000 up to several hundreds of thousands of copies per ml of whole blood. These values may be confused with those resulting from ciHHV-6. Indeed, in the few individuals who have ciHHV-6, HHV-6 DNA is present in every nucleated cell, in particular cells from hair follicles, the CNS, and peripheral blood. In ciHHV-6 subjects, HHV-6 DNAemia in whole blood usually exceeds 1 million copies per ml, which is far beyond the values observed in most active infections (120, 132). However, when the white blood cell count is significantly decreased, as observed in hematopoietic stem cell transplant (HSCT) recipients, the results for absolute viral loads in blood may be ambiguous. In this case, the expression of results as numbers of copies per cell, with values of 1 or more, may help to distinguish ciHHV-6 from most active infections, in which the average viral load per cell is usually much lower (133). The use of droplet digital PCR may also be valuable for the identification of ciHHV-6 by precisely determining the ratio of HHV-6 DNA to cellular DNA without the use of a standard curve (126, 134).

In the case of active infection limited to a specific body compartment, viral loads in whole blood and in the concerned compartment (bodily fluid, cell fraction, or organ biopsy specimen) have to be compared with each other in order to estimate the respective contributions of blood input and *in situ* virus multiplication. In this situation, it is admitted that the value of viremia does not need to be significantly high to establish the diagnosis of active infection. Accordingly, due to the sensitivity and specificity of current PCR techniques, the detection of HHV-6 DNA in CSF is considered sufficient for the diagnosis of an active infection of the CNS, regardless of the level of viremia (135, 136). This raises the question of possible restricted local reactivations in the CNS, as suggested in some cases for HHV-6 encephalitis after HSCT (137, 138). In this context, the quantification of HHV-6 DNA in CSF, instead of its simple qualitative detection, may be worthy, particularly in cases of longitudinal follow-up. In ciHHV-6 patients, unusually large numbers of HHV-6 DNA copies are observed in body fluids, which may be interpreted falsely as acute infection in these body compartments: this has been described in particular for CSF, with the risk of misdiagnosing CNS infections (139).

The detection and/or quantification of transcripts specific to a productive viral cycle, for instance, late gene transcripts, would be another way to characterize active infections. Several requirements have to be satisfied for that purpose, namely, the specificity of target mRNAs as markers of active infection in any cell or tissue, the definition of clear cutoff values for quantitative analyses, and the standardization of quantification procedures. For now, these criteria are not fulfilled. The same comment can be made regard-

ing the detection of specific virus-encoded proteins by means of their antigenic properties or by mass spectrometry techniques (140). Moreover, detection procedures based on proteins raise the additional challenge of a reduced sensitivity compared with that of studies of nucleic acids.

Latent Infection

Latent HHV-6 infection corresponds to the widespread chronic infection classically described for all human herpesviruses, although its molecular definition at the cellular level is far from clear and cannot be restricted solely to episomal persistence in the absence of any gene expression (see above). It also corresponds to the existence of ciHHV-6, which is present in only a minority of the general population. In the latter case, the potential pathogenic effects of HHV-6 may affect any tissue and organ, far beyond the usual main locations of community-acquired infection, which particularly involves lymphoid tissue and the CNS. Concerning the clinical syndromes possibly related to latent infection, our current understanding of the disease mechanism is extremely limited, but it hypothetically tends to invoke discrete expression of viral genes, an inflammatory response, and immune dysfunction rather than a modest but present HHV-6 multiplication.

Seropositivity for HHV-6 in the absence of active infection markers thus may appear to be a convenient marker for latent infection. However, there are limitations of serologic assays, as mentioned above. In addition, the production of serum antibodies in some ciHHV-6 individuals is affected, presumably through a phenomenon of immune tolerance, which constitutes an additional challenge for diagnosis (66). As indicated previously, the presence and amount of U94 transcripts might reflect episomal latency and, in some cases, ciHHV-6. Moreover, a recombinant U94 protein has been used to create an ELISA that would be suitable for exploring the antiviral immune response in HHV-6-related chronic diseases (141). However, the relevance of U94-related markers remains to be demonstrated formally.

The spectrum of other virological findings observed during *in vivo* latent infection is theoretically wide but practically unknown. Except for the detection of HHV-6 DNA in ciHHV-6 subjects, the major hindrance for the detection of direct viral markers is their intrinsically low level of expression. In that context, indirect markers related to HHV-6-related immune responses may reveal interesting features, provided that studies are founded on relevant pathophysiological hypotheses, large human populations, and numerous controls. Accordingly, particular attention has been paid to viral factors potentially involved either directly or indirectly in chronic diseases, in particular those presumably stimulating inflammation, cell transformation, and autoimmunity processes.

The association of HHV-6 with cell transformation and cancer has been a debated question since the early times of virus discovery (142). HHV-6 has direct transforming capacities *in vitro*, which have been mapped to the DR7 gene, also designated ORF1 (73, 143). As mentioned previously, the virus can transactivate the expression of other viruses, such as EBV and human herpesvirus 8 (HHV-8), which are truly oncogenic viruses (144, 145). In addition, the special context of ciHHV-6 may offer the opportunity to express specific HHV-6 genes in particular cells which do not express virus receptors and usually escape from exogenous infection. However, to date, no report has mentioned an increased frequency of malignancies in ciHHV-6 subject populations. Finally, the ability of HHV-6 to promote oncogenesis even in the absence of direct transforming activity may rely on its inflammatory properties, as discussed above. This provides an incredibly large number of cellular signaling pathways to explore. Similarly, concerning autoimmunity, multiple mechanisms involving HHV-6 either directly or indirectly have been hypothesized (142). A molecular mimicry which might result in cross-reactivity between self and viral epitopes has been found between the sequences of the U24 protein and myelin basic protein (146). The reactivity of HHV-6-specific T cells against infected cells, as investigated *in vitro* by lymphoproliferative responses to viral antigens, may induce a bystander activation or damage of uninfected cells present within the same microenvironment. The interaction of HHV-6A with its CD46 receptor, which is a regulator of complement activation, may cause an upregulation of this process, including deleterious lytic cell effects and an increase of the soluble form of CD46 (147). The dysfunction of HHV-6-induced immune responses leading to autoimmune processes would involve not only specific T cell responses but also NK cell-mediated activation and killing (148). All these phenomena would converge to promote release of self antigens and polyclonal activation.

HHV-6 Species

It is now highly recommended to identify the causative HHV-6 species as HHV-6A, HHV-6B, or a mixture of both when a diagnosis of HHV-6 infection is made (22). Knowing the causative HHV-6 species precisely has no impact on diagnosis or management at present. Nevertheless, this information will prove valuable in defining the spectrum of diseases associated with each of the viruses and in the event that virus-specific therapeutic options become available. Any HHV-6 isolate can be classified unambiguously as HHV-6A or HHV-6B by using diverse methods based mainly on PCR and/or nucleotide sequencing. Over 2 decades, many publications concerning *in vivo* studies have neglected this differentiation step, which is still not possible using conventional serologic assays. As a result, our knowledge of the specific epidemiology and pathophysiology of each HHV-6 species is far from complete. Concomitant or sequential infections with the two species can occur, as well as simultaneous infection with two different strains of the same species, which increases the complexity of this analysis. As a whole, HHV-6B is by far the most frequently detected species in peripheral blood, saliva, and CSF, both for asymptomatic infections and for diseases potentially associated with HHV-6 (3, 9, 121, 149). In most populations studied, HHV-6B also appears to be the first species acquired early in life and the quasi-exclusive agent of exanthema subitum, the prototypic disease associated with HHV-6 primary infection (see below) (3, 9). Whether that predominance of HHV-6B detection is due to technical and physiological constraints or reflects a real higher involvement of HHV-6B over HHV-6A within human HHV-6 infections remains an enigma.

Other Favoring and Confounding Factors

Immunosuppression is the major factor promoting endogenous HHV-6 reactivations, so this virus is a true opportunistic pathogen (3, 135, 150). Such pathogenicity has been observed most frequently in situations in which the functions of cell immunity are impaired, in particular in HSCT and solid organ transplant (SOT) recipients as well as HIV-infected individuals. Except for the physiological loss of maternal immunity, which opens the way

TABLE 2 Clinical syndromes consistently (C) or hypothetically (H) associated with HHV-6 infections

Stage of HHV-6 infection	Disease or symptom[a]
Primary infection (congenital)	Abnormalities at birth and during immediate postnatal period (H)
	CNS developmental defects (H)
Primary infection (postnatal)	Exanthema subitum (roseola infantum, sixth disease) (C)
	Fever, seizures (C)
	Mild gastrointestinal and respiratory tract symptoms (C)
	Thrombocytopenia, infectious mononucleosis-like syndrome (C)
	Hepatitis, gastroenteritis, colitis (C)
	Meningoencephalitis and encephalitis (C)
	Hemophagocytic syndrome (H)
	Temporal lobe epilepsy (H)
Reactivation (and possible reinfection)	Fever (C)
	Rash (C)
	Thrombocytopenia, leukopenia, anemia, bone marrow suppression (C)
	Hepatitis (C)
	Encephalitis, neurocognitive dysfunction (C)
	Retinitis (C)
	Pneumonitis (C)
	Drug-induced hypersensitivity syndrome (C)
	Gastroenteritis, colitis (H)
	Temporal lobe epilepsy (H)
	Allograft rejection (H)
	Graft-versus-host disease (H)
	Thrombotic microangiopathy (H)
	Higher incidence and severity of infections with HCMV, fungi, and other opportunistic pathogens (H)
Chronic latent infection (with possible sporadic reactivations)	Multiple sclerosis (H)
	Hashimoto's thyroiditis (H)
	Myocarditis, cardiomyopathy (H)
	Chronic fatigue syndrome (H)
	Acceleration of evolution to AIDS in HIV-positive individuals (H)
	Hodgkin's disease (H)

[a] HCMV, human cytomegalovirus; C or H, consistent or hypothetical association with HHV-6 (keeping in mind that robust large-scale studies are lacking in most cases, which makes this classification prone to frequent changes and subject to personal interpretation).

to primary infection during infancy, no other circumstances, including pregnancy and long-term corticosteroid therapy, have been reported to favor active HHV-6 infection. Conversely, it is worth mentioning that HHV-6 reactivations can be detected in immunocompetent healthy individuals, as evidenced by elevated HHV-6 DNA loads in blood and saliva in the absence of any ciHHV-6 (9, 151, 152).

As mentioned previously, HHV-6 itself is presumably an immunomodulatory virus that is capable of complex interactions with the immune system and other pathogens, particularly HCMV (150). These intricate interactions may induce immune dysfunctions, which may be translated into specific diseases appearing, at least in part, to be indirect effects of HHV-6 infection (Table 2). As examples, HHV-6 reactivations boost the activity of the immune system, which may lead to graft rejection in transplant recipients, hypersensitivity syndrome in the context of exposure to particular drugs, or, in contrast, enhancement of HCMV disease (150).

In addition to sequential infections of most subjects by HHV-6B and HHV-6A, the existence of exogenous HHV-6 reinfections can be speculated from reports of intraspecies recombination and dual infection with two distinct strains of the same species (3, 29, 36). However, it is not known whether these exogenous reinfections are characterized by a particular pattern regarding replication dynamics, serologic responses, establishment of latency, and putative interactions with ciHHV-6 compared with endogenous reactivations. In order to clarify these questions, the design of future investigations of active infections may include not only the identification of the causative HHV-6 species but also the precise characterization of the involved strain by means of molecular epidemiology techniques.

CLINICAL IMPACTS OF HHV-6 INFECTIONS

Primary Infections

Since the initial report in 1988, converging data have proven that HHV-6 primary infections cause acute febrile diseases in young children of 6 months to 3 years of age, with the most emblematic one being exanthema subitum (also known as roseola infantum or sixth disease) (20, 78, 153). Other, less typical combinations of fever, seizures, skin rash, and gastrointestinal and respiratory tract symptoms have been reported, with most of them leading to a quick, favorable outcome (Table 2). As a whole, HHV-6 primary infections account for 10 to 20% of febrile illnesses at this age, and the majority of these infections are associated with clinical symptoms, in disagreement with the previous assumption that the majority of these infections are asymptomatic (9, 80). The causative relationship between active HHV-6 infection and disease has been established from the temporal association between the following clinical and virological findings: evidence of viremia, demonstrated first by means of virus isolation and next by PCR, at the acute phase of illness; presence of HHV-6 DNA in the CSF of children with seizures, pointing to direct infection of the CNS; and seroconversion observed after recovery (3). For still unknown reasons, the clinical symptoms might differ slightly according to country: the rate of exanthema subitum associated with primary infection seems to be higher in Japan than in the United States (153). As mentioned above, the responsible variant was found to be HHV-6B in most cases. Primary infections with HHV-6A have been reported much less frequently, and it is generally assumed that HHV-6A is acquired after HHV-6B, through an asymptomatic infection. However, symptomatic primary infections with HHV-6A have been described for children in the United States as well as in sub-Saharan Africa (154–156).

Infrequently, primary infection is associated with a more severe disease, such as hepatitis, including fulminant forms in some cases, thrombocytopenia, infectious mononucleosis-like syndrome, hemophagocytic syndrome, gastroenteritis, colitis, or myocarditis (3, 9, 153).

Neurological complications include meningoencephalitis and encephalitis, which represent ultimate stages of CNS involvement during primary infection and whose outcomes remain uncertain (6, 9, 78). A nationwide survey in Japan showed an unexpectedly poor prognosis for exanthema subitum-associated encephalitis (157). In addition, two ultimately fatal cases of encephalopathy

following acute HHV-6 infection were recently reported for two children with underlying genetic mitochondrial disorders (158). As in benign forms of CNS involvement, the recognition of a concomitant active infection permits the etiological diagnosis and is founded on a combination of virological parameters which include the finding of HHV-6 DNA in blood, saliva, and CSF, as well as specific seroconversion and IgM detection (3, 136). The question of active virus replication has been more difficult to address in considering cases of mesial temporal lobe epilepsy which have been related to HHV-6B in children: there was no evidence of systemic viral infection, but high levels of HHV-6 DNA were detected in the hippocampal regions following temporal lobectomy, suggesting an active local multiplication of the virus, possibly focused in astrocytes (6, 42). The role of HHV-6 in febrile status epilepticus, putatively related to encephalitis, was addressed among 169 children in the context of a multicenter prospective study: 54 children (32%) were found to have HHV-6B viremia, while none had HHV-6A infection, and no HHV-6 DNA was detected in any CSF specimen, keeping the question of subsequent development of hippocampal sclerosis and temporal lobe epilepsy under investigation (78, 159). HHV-6 primary infections seem infrequent among adults due to the nearly universal occurrence of this event in early childhood. The question is whether clinical manifestations are usually as benign as those observed in most children. The diagnosis of encephalitis associated with primary infection is less likely to be suspected in this context, whereas at least one case of mononucleosis-like syndrome has been reported for an adult (83, 160).

Congenital HHV-6 infection following primary infection of the embryo or fetus during pregnancy has been found to occur in about 1% of children, a frequency close to that observed for HCMV. In contrast with the case for HCMV, few data are available concerning associated diseases. No symptomatic early infection has been reported, even for children who exhibit detectable active infection at birth (9, 82). However, it was recently reported that HHV-6 congenital infection was associated with lower scores on the Bayley scale of infant development II MDI at 12 months of age, which requires further confirmation (161). Congenital infection results from either the germ line transmission of ciHHV-6 (i.e., congenital ciHHV-6) or transplacental passage of the virus following endogenous reactivation (or putative exogenous reinfection) in the mother. Unexpectedly, in view of its low frequency in adults, ciHHV-6 has been found to be the predominant context associated with congenital HHV-6 infection (162, 163). In addition, HHV-6A was detected significantly more frequently in congenital infection than in postnatally acquired infection, and in a study limited to 6 children with transplacentally acquired infection, all the mothers had ciHHV-6. These data have led to a picture of congenital infection which appears to be far more complex than that of HCMV and in which ciHHV-6 plays a prominent role (162, 163). These results also raise numerous questions, which mainly refer to the capability of ciHHV-6 to feed virus reactivation, tolerate exogenous HHV-6 reinfection, induce protective immunity, and enhance potential developmental deficits, particularly in the CNS.

Reactivations and Reinfections

Active HHV-6 infections often remain totally asymptomatic. Together with the fact that HHV-6 infections are ubiquitous and highly prevalent, this explains the difficulty in unambiguously establishing the causative role of the virus in clinical manifestations concomitant with active infections. However, the following elements may help to demonstrate or at least cause one to suspect the responsibility of HHV-6 in a given disease: a favoring condition, such as a defect of cellular immunity; the temporal convergence between the clinical events and dynamics of viral replication; the correspondence between the nature of symptoms and virus tissue tropism; the absence of any other pathogen known to be a cause of the disease; and the consistency of association between active infection and disease in a similar context.

The clinical symptoms associated with HHV-6 reactivations in transplant recipients appear to occur in a minority of patients but are involved in a wide spectrum of syndromes (164–166). Some symptoms may be considered nonspecific, such as fever, rash, and transiently decreased numbers of circulating blood cells belonging to the granulocyte/macrophage, erythroid, and megakaryocytic lineages (Table 2). In contrast, subacute limbic encephalitis and delayed engraftment are now recognized as typical opportunistic diseases due to HHV-6 reactivation in HSCT recipients (135, 150, 160). These patients, particularly those receiving allogeneic transplants, are at high risk of developing a reactivation within the first 4 weeks after cell transfer and, subsequently, suffering a life-threatening illness concerning the CNS and/or bone marrow, two well-known sites of HHV-6 latency. However, encephalitis develops in only a small proportion of patients experiencing HHV-6 reactivation. HHV-6 encephalitis is usually diagnosed by the detection of viral DNA in CSF, HHV-6 viremia, and abnormal magnetic resonance imaging findings in temporal lobes, with the onset of symptoms occurring at the end of the first month after transplant, on average. The majority of cases are due to HHV-6B. Additional evidence for the direct effect of HHV-6 on the CNS came from a study which showed a strong correlation between HHV-6 reactivation and CNS dysfunction as measured by delirium and neurocognitive decline in HSCT patients (167). Bone marrow suppression in HSCT patients is the other major complication associated with HHV-6 reactivation, which may evolve to secondary graft failure. Other serious illnesses, such as graft-versus-host disease, thrombotic microangiopathy, HCMV reactivation, and gastrointestinal disease, have been found to be associated with HHV-6 reactivation, but the causative relationship is less clear and warrants further investigations.

HHV-6 reactivations have also been detected frequently in SOT patients, but with different rates according to the characteristics of the transplanted organ, the nature of immunosuppressive therapy, and the administration of prophylactic anti-HCMV treatment, which is presumably also active against HHV-6 (3, 150, 168). HHV-6B again remains the main species detected. Concomitant febrile episodes and other nonspecific symptoms, such as leukopenia and thrombocytopenia, have often been observed (164). Specific severe diseases have been reported for SOT patients, but the causative relationship with HHV-6 does not appear to be as convincing as that for HSCT patients. In renal transplant recipients, the spectrum of clinical manifestations seems limited, whereas hepatitis, pneumonitis, bone marrow suppression, and encephalitis have been reported for liver, lung, and heart transplant patients (168, 169). However, a synergistic pathogenic role of HCMV could not be ruled out in all cases. The role of HHV-6 in graft rejection remains a debated question, as is the facilitation of superinfections with fungi and other opportunistic pathogens (7, 164).

For HIV-positive patients, the clinical impact of HHV-6 reactivation was considered an important question before the development of antiretroviral therapy, in parallel with the major pathogenic role observed for HCMV in this context. An increased frequency of active infections was observed in late stages of the course of AIDS but was questioned for earlier stages (170, 171). As a whole, the capability of HHV-6 to accelerate the progression to AIDS remains controversial, while its opportunistic role among AIDS-associated infections was convincingly demonstrated by reports of cases of encephalitis, pneumonitis, and retinitis (172–175).

Drug-induced hypersensitivity syndrome (also known as drug rash with eosinophilia and systemic symptoms [DRESS]) appears to often be associated with active HHV-6 infection (176–178). The disease is constituted of severe adverse drug reactions associated to various degrees with skin rash, fever, lymph node enlargement, liver dysfunction, and blood leukocyte abnormalities. The strength of the association between virus and disease has been recognized by authors from Japan, who have included HHV-6 active infection in the list of criteria used for the diagnosis of this syndrome, a proposal which is not yet universally accepted (179). The point is to understand how active viral infection interacts with drug exposure and immune dysfunction to generate the disease. The possible role of EBV, another human herpesvirus, in this syndrome has also been suggested, which enables the proposal of a scenario founded on herpesvirus reactivation (179, 180). The starting event would be the triggering of virus multiplication by the drug, thus resulting in immune activation by viral antigens and extensive antiviral T cell responses at the origin of the disease. Therefore, it is tempting to speculate that a similar mechanism is possible for HHV-6, a hypothesis supported by the finding that drugs inducing the syndrome, such as amoxicillin and sodium valproate, are capable of directly stimulating HHV-6 replication (4, 181, 182).

Chronic Infections

Numerous challenges arise in considering the chronic clinical manifestations that can be related either directly or indirectly to the persistence of HHV-6 in the body, apart from or independent of clear-cut episodes of active viral infection. This definition makes the demonstration of HHV-6 as the cause of these diseases extremely difficult. Again, the main obstacle comes from the fact that HHV-6 infection occurs early in life, is nearly universal, and is lifelong. Therefore, many of the criteria essential for evaluating causality are lacking, in particular the specificity and temporality of associations between the virus and symptoms. In addition, the current limitations of investigational tools concern the interpretation of results regarding viral loads, transcripts, and immune markers, as well as the low level of viral expression during latent infection, the existence of two HHV-6 species, and the lack of relevant animal models.

The role of HHV-6 as a possible trigger for multiple sclerosis (MS), an inflammatory demyelinating disease of the CNS, has been debated for a long time (6, 183, 184). MS is an autoimmune condition whose pathogenesis seems to be multifactorial, with numerous environmental factors being suspected to act in the genesis, development, and relapses of the disease. Among them, HHV-6 appears to be one of the most serious candidates in the category of infectious triggers, on the basis of immunological, virological, and experimental data (6). These data include the isolation of this virus from diseased CNS tissues of MS patients, serologic studies of HHV-6 antibody reactivity in their sera and CSF, and *in situ* detection of HHV-6 DNA transcripts (141, 183, 185–188). This hypothesis also results from general considerations founded on the neurotropism of the virus combined with its theoretical capacity to induce both neuroinflammation and autoimmunity. However, to date, no definite proof of this causative role has been provided (184). Such a proof would open the way to novel MS therapies based on specific antiviral effectors.

Hashimoto's thyroiditis, also known as chronic lymphocytic thyroiditis, is another autoimmune disease in which viral infections are suspected to act as environmental triggers (10). A recent study suggested the putative role of HHV-6A infection (148). The virus was detected more frequently in thyroid cells of patients suffering from Hashimoto's thyroiditis than in those of control patients, such as patients with Graves' disease or multinodular goiter. This study also provided evidence that HHV-6-infected thyroid cells were susceptible to NK cell-mediated cell killing, suggesting a possible mechanism for autoimmunity induction.

The association of HHV-6 with cardiac and vascular diseases has been suggested by several authors. HHV-6 has been listed among the viruses which can cause myocarditis and subsequent chronic cardiomyopathy, as reflected by the frequent detection of the virus in heart muscle biopsy specimens (7, 189–191). HHV-6 is capable of infecting vascular endothelium both *in vivo* and *in vitro*, which might support a role in the genesis of diseases affecting coronary and peripheral arteries (47, 192). These effects might be strengthened by the inhibition of angiogenesis induced by U94 gene expression (75).

Chronic fatigue syndrome is a chronic illness causing a major functional impairment whose pathogenesis is incompletely understood (193). The underlying biological abnormalities include markers of chronic immune activation as well as neuroendocrine dysfunction. The possible role of HHV-6 in this syndrome is supported by many studies demonstrating an increased rate of viral reactivation events, as shown by PCR studies of plasma, serum, and CSF (194–196). As expected, the data referring to HHV-6 immune responses are more conflicting. Again, the neurotropism of the virus and its capacity to dysregulate inflammatory responses are indirect arguments for considering its role in the disease but do not appear to be sufficient to recommend HHV-6 therapy in this context.

Regarding malignancies, a putative role of HHV-6 has been discussed for Hodgkin's disease in parallel with the accumulation of data which incriminate EBV (5, 7). This particularly concerns the nodular sclerosis form of the disease in young adults (143, 197). Note that recent results found no association between ciHHV-6 and classical Hodgkin's disease (198). As far as other lymphoproliferative diseases are concerned, a hypothetical role of HHV-6 in the pathogenesis and progression of angioimmunoblastic T cell lymphoma is supported by a higher detection frequency and load of HHV-6 DNA in tumor tissue than those in other lymphomas (199, 200).

TREATMENT OF HHV-6 INFECTIONS

Antiviral Agents

Three drugs initially developed to target HCMV infection have been shown to be efficient against HHV-6 infection both *in vitro* and *in vivo*: ganciclovir, foscarnet, and cidofovir (Table 3). Acy-

TABLE 3 Compounds showing effectiveness against HHV-6

Drug[a]	Prodrug	Chemical structure	HHV-6 target	EC_{50}[b] (μM)	Clinical use	Reference(s)
Ganciclovir	Valganciclovir	Nucleoside analogue	DNA polymerase	2.0–68.6	Yes (approved for HCMV)	203, 204
Foscarnet	None	Pyrophosphate analogue	DNA polymerase	6–86	Yes (approved for HCMV)	203, 204
Cidofovir	Brincidofovir	Nucleotide analogue	DNA polymerase	0.3–46	Yes (approved for HCMV)	203, 204
H2G	Valomaciclovir	Nucleoside analogue	DNA polymerase	36.6	Yes (clinical trials)	214–216
Cyclopropavir	6-Deoxycyclopropavir	Nucleoside analogue	DNA synthesis step	0.5–7	No (pharmaceutical development)	203, 204, 217–219
S2242	None	Nucleoside analogue	DNA polymerase	0.02–0.03	No (pharmaceutical development)	216, 220
3-Deaza-HPMPA	None	Nucleotide analogue	DNA polymerase	1.2–1.4	No (discontinued development)	220
CMV423	None	Indolizinecarboxamide	Tyrosine kinase[c]	0.017–0.053	No (discontinued development)	203, 204, 222
Artesunate	None	Semisynthetic derivative of artemisinin	Sp1/NF-κB activation pathways[c]	3.8	Yes (approved for malaria)	212, 213

[a] No drug is officially approved for use on HHV-6.
[b] EC_{50}, 50% effective dose (range or unique value, according to the data in referenced studies).
[c] Cellular component target.

clovir, the emblematic antiviral drug against herpes simplex virus and varicella-zoster virus infections, is active against HHV-6 *in vitro*, but only at very high concentrations. These concentrations cannot readily be obtained in body fluids *in vivo*, which explains why HHV-6 has to be considered naturally resistant to this agent (201). The three efficient anti-HHV-6 compounds exhibit the same inhibition activity against both HHV-6A and HHV-6B *in vitro* (3, 202–204). The mechanisms of this antiviral activity are similar for both HCMV and HHV-6: the viral DNA polymerase is specifically inhibited by the triphosphorylated form of ganciclovir, the diphosphorylated form of cidofovir, and foscarnet, in the latter case without any chemical modification.

The first phosphorylation step of ganciclovir is catalyzed by a protein kinase, encoded by the HCMV UL97 or HHV-6 U69 gene, while the further phosphorylation steps of ganciclovir and the two phosphorylation steps of cidofovir are dependent upon the activity of cellular kinases. In agreement with this mechanism of action, acquired virus resistance to these drugs has been related to mutations of target viral genes involved in drug susceptibility for both HCMV and HHV-6: protein kinase and DNA polymerase genes in the case of resistance to ganciclovir and the DNA polymerase gene only in the case of resistance to foscarnet and cidofovir (123, 205–208). Accordingly, the risk of simultaneous resistance of different herpesvirus species to the same drug from the concomitant selection of mutations in homologous genes has to be considered. This was illustrated by a report of HHV-6 resistance to ganciclovir following prolonged treatment of HCMV infection with this drug (205). HHV-6 and HCMV do not express any thymidine kinase, the enzyme which performs the first phosphorylation step for other antiherpetic drugs, such as acyclovir, penciclovir, and brivudin. This explains in part the low activities of those drugs against HCMV and HHV-6.

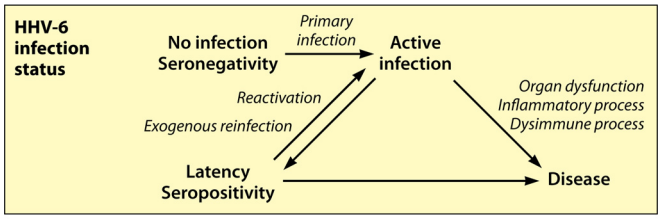
In addition to their cost and the possibility of selecting drug-resistant viruses, efficient anti-HHV-6 drugs exhibit another drawback which may restrict their use in patients: despite their selectivity with respect to viral enzymes, they exhibit a certain degree of toxicity for human cells and organs, i.e., bone marrow in the case of ganciclovir and the kidneys in the case of foscarnet and cidofovir. The usefulness of these antivirals against diseases thought to be caused by HHV-6 has been reported for humans, but only in the context of uncontrolled studies (5). Therefore, the assumed efficacy of these drugs *in vivo* is not formally demonstrated, and none is officially approved for treatment of HHV-6 infection. Similarly, the possible synergistic activity of these compounds administered together, for instance, ganciclovir and foscarnet, as previously demonstrated for HCMV, has not yet been proven for HHV-6.

Other nucleoside or nucleotide analogues are potentially efficient drugs, particularly some phosphonate derivatives which are currently under preclinical development or in early clinical trials (203, 209, 210). Brincidofovir, also known as CMX001, is an orally administered lipid-ester derivative of cidofovir which is active against numerous DNA viruses, including HHV-6, is detected at significant levels in CSF from treated patients, and is less toxic for the kidney than cidofovir (137, 204, 211). Artesunate is a derivative of artemisinin, a drug used for the treatment of malaria. It has been proven to inhibit the replication of several human herpesviruses *in vitro*, in particular HCMV and HHV-6, and has been given to treat HCMV infections *in vivo* as a compassionate use. Its mechanism of antiviral activity likely involves the modulation of cellular activation pathways involving Sp1 and NF-κB (212, 213). Valomaciclovir, a drug developed initially against varicella-zoster virus, has also shown significant activity against HHV-6 *in vitro* (214–216). Other antiviral compounds have demonstrated promising experimental anti-HHV-6 activity, including the nucleoside/nucleotide analogues SS2242, A-5021, cyclopropavir, and 3-deaza-HPMPA and the non-nucleoside inhibitor CMV423 (4, 217–222). These are all still at a step of preclinical development.

Recently, the complementary use of immunotherapy, particularly in the context of HSCT, has been considered through the generation of polyclonal cytotoxic T lymphocytes targeted to several opportunistic viruses, including HHV-6 (100, 203). The concept of an adoptive therapy based on these T cells is very attractive, due to the possibility of circumventing drug cytotoxicity and resistance, but requires validation in ongoing clinical trials.

Indications and Management of Clinical HHV-6 Treatments

Independently of any antiviral drug administration, a key factor for the control of active HHV-6 infection is the reversal of immunosuppression when this favoring factor is present and accessible to medical modulation. Thus, reducing the dose of immunosuppressive drugs is a valuable approach to consider when this option is available (11, 223).



Diagnosis			
Clinical symptoms	–	–	+
Virological findings	±	++	+++

Therapy	Prophylactic	Preemptive	Curative
N° of treated pts	+++	++	+
Risk of side effects and resistance	+++	++	+
Costs	+++	++	+

FIG 2 Overview of the three general options for the treatment of HHV-6 infection. The "+" and "−" symbols indicate the presence, absence, and/or level of the indicated parameter.

The general strategy of antiviral therapy against HHV-6 may be inspired by the current practices of anti-HCMV treatment, thus leading to a choice between prophylactic, preemptive, and curative approaches (Fig. 2). The essential target is active infection, since the available drugs are not efficient against the potential deleterious effects of latent infection. Hence, virological findings provide valuable information in order to define the most convenient strategy. Prophylactic treatment has the advantage of totally protecting at-risk individuals from the deleterious effects of active HHV-6 replication by preventing either primary infection (mainly for children) or reactivation (for most adults). However, this strategy requires the treatment of a large number of individuals, for instance, virtually all immunocompromised patients; induces high costs; and exposes individuals to numerous side effects as well as to emergence of resistance. Conversely, curative therapy is initiated once HHV-6-associated disease has been diagnosed, which concerns fewer people but exposes them to the risk of therapeutic failure due to late intervention. Preemptive therapy is the median solution, with the detection of viral replication at a significant level being the starting signal for therapy prior to any declared disease. Its cost-effectiveness is presumably favorable, but its indications require a precise analysis of HHV-6 replication dynamics. Note that regarding HCMV infection, the early detection of virus reactivation by use of PCR-based molecular tools has permitted successful preemptive therapy, particularly in transplant recipients (122).

It is tempting to speculate on the following items as possible additive criteria for initiating therapy: (i) demonstrated active infection with a significantly elevated viral load in the absence of ciHHV-6; (ii) immune suppression context; (iii) concomitant clinical symptoms relating to virus replication on a pathophysiological basis; and (iv) absence of any other causative agent, in particular HCMV, but keeping in mind that ganciclovir, foscarnet, and cidofovir are also active against HCMV infection. As indicated previously, to date, the nature of the HHV-6 species, either HHV-6A or HHV-6B, has not been considered a key feature for treatment decisions. Taking all available data together, it currently seems premature to propose a prophylactic therapy in order to prevent HHV-6 reactivations or a preemptive therapy of virologically proven active infection in order to prevent clinical manifestations. The spontaneous evolution of HHV-6 reactivations and their association with disease are not sufficiently understood to enable such therapeutic strategies. It is obvious that active HHV-6 infections are spontaneously controlled in many circumstances, which emphasizes the risk of giving a treatment without any need. Accordingly, the International Herpesvirus Management Forum and the American Society of Transplantation do not recommend antiviral prophylaxis for HHV-6 infection but promote the initiation of antiviral therapy in case of HHV-6 encephalitis. Intravenous ganciclovir and foscarnet are proposed as first-line drugs (164, 223, 224). However, no other recommendation has been approved internationally for the therapy of active HHV-6 infections while numerous questions are pending. Those questions refer to the precise timing for therapy initiation, the preferred drug to be used, and treatment duration. An additional question is that of maintenance therapy administered in order to prevent relapses after the acute disease has been controlled.

Indeed, the limbic encephalitis of HSCT patients fulfils most of the criteria listed above and obviously is a potential target for antiviral drugs. The severe CNS diseases associated with primary infection in children might be another priority indication, but the data supporting this assumption need to be strengthened. As a general comment, in the case of CNS disease associated with HHV-6 infection, ganciclovir and foscarnet, either alone or in combination, might be preferred over cidofovir, as the ability of the latter drug to penetrate the blood-brain barrier in humans is controversial (225). No study has yet been performed to define the specific dosing and duration of antiviral therapy. Therefore, the patterns of CMV therapy are used in most cases, with an approximate duration of 3 to 4 weeks (165).

Whatever the indication, the effectiveness of antiviral therapy must be monitored by the concomitant regression of clinical symptoms and decrease of viral load, as performed to assess the response to anti-HCMV treatments (122). A divergence between the evolution of disease and that of viral parameters would lead one to suspect a causative agent other than HHV-6 or a pathogenic mechanism distinct from direct effects of active infection. In addition, a therapeutic failure following the prolonged administration of an efficient antiviral drug may lead to suspicion of the emergence of a resistant virus and should encourage performance of a drug susceptibility test based on a phenotypic assay or a genetic analysis (226). The resistance of HHV-6 to antiviral drugs is not currently as crucial a question as it is for HCMV (122). This weak concern about resistance is correlated with the present limited use of antivirals against this virus. However, the risk of HHV-6 resistance resulting from a previous exposure to drugs in the context of other concomitant herpesvirus infections, notably HCMV infection, does exist and must be kept in mind (205).

For any indication, the validation of therapeutic options against HHV-6 infections will require randomized controlled therapeutic trials, the only valuable strategy for establishing formal treatment guidelines (203).

PENDING QUESTIONS AND PERSPECTIVES

Deciphering the Clinical Impact of Chromosomal Integration

In addition to the difficulties in interpreting results for nucleic acid testing diagnosis, ciHHV-6 raises numerous theoretical clin-

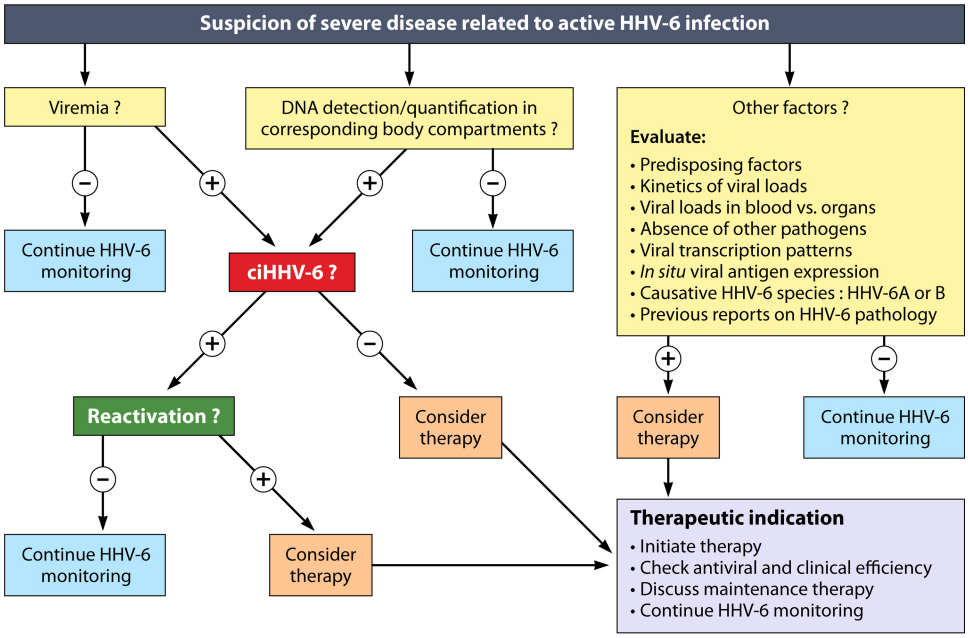


FIG 3 Hypothetical algorithm for diagnosis and therapy decisions in a situation of severe disease potentially associated with active HHV-6 infection.

ical concerns related to virus reactivation, dysregulation of immune responses, and instability of the cell genome. If proven, these phenomena might significantly alter the frequencies of cancer and inflammatory, autoimmune, or drug-induced hypersensitivity diseases in ciHHV-6 subjects. They also might modify the rate of side effects and the overall prognosis of transplantation and transfusion for patients receiving ciHHV-6 blood cells or organs (66). Therefore, further research is needed to clarify these points and to provide clear information for persons carrying ciHHV-6. This can be done by means of retrospective studies in order to determine whether ciHHV-6 is significantly more frequent in some diseases. Alternatively, prospective studies of cohorts of ciHHV-6 individuals will analyze the occurrences of particular illnesses compared to those for cohorts of control subjects.

Differential Pathogenic Roles of HHV-6A and HHV-6B

HHV-6A and HHV-6B are now defined as distinct virus species (22). Their epidemiologies are also suspected to differ from each other regarding the time of virus acquisition in life, their role in HHV-6 primary infection according to geographic region, their distribution in human tissues, and their modes of human-to-human transmission. Major questions refer to disease associations. Some reports have suggested an increased severity of HHV-6A over HHV-6B, particularly in acute and chronic CNS diseases suspected to be linked with HHV-6 infection. However, only HHV-6B has been found to be associated with mesial temporal lobe epilepsy and status epilepticus, while only HHV-6A has been associated with Hashimoto's thyroiditis (148, 159, 227). It is now essential to make a clear distinction between both species in all research projects regarding HHV-6 epidemiology and pathogenicity. This distinction might provide dramatic insights into our understanding of the various infectious events that are collectively designated HHV-6 infection. This, in turn, would enable us to define specific diagnostic and treatment strategies on a more relevant basis.

Causative Role of HHV-6 in Chronic Diseases

The association of HHV-6 with chronic diseases has been supported by *in vivo* immunological and molecular findings as well as experimental data from cell models. However, the convincing demonstration of a causative role of HHV-6 in diseases such as MS, Hashimoto's thyroiditis, and chronic cardiomyopathy, either as an initial inducer or as a further trigger, is a huge challenge. The ubiquitous distribution and lifelong duration of HHV-6 infection create major obstacles for case-control and/or prospective studies. Clinical studies based on the potential beneficial effects of anti-HHV-6 drugs or immune effectors in these diseases have very limited options. These limitations are due to the small number of available virus inhibitors, the huge number of parameters to be investigated, and the long duration of experimental therapy, which may expose treated patients to numerous side effects. In addition, complex chronic illnesses may rely on multiple pathogens which are also sensitive to the administered antiviral treatments, such as HCMV and other herpesviruses. This makes the interpretation of therapy effects difficult regarding the possible etiological role of HHV-6.

Algorithms and Priorities for HHV-6 Treatments

Despite the uncertainties regarding HHV-6 pathogenicity and the limitations of therapeutic options, it would be worth setting up algorithms with the double purpose of improving the knowledge about HHV-6 infection and its overall prognosis in patients. The situation which currently appears to be the most favorable for the application of such algorithms is that of active severe HHV-6 infections (Fig. 3). There are many reasons for this. Active HHV-6 replication can be diagnosed confidently and quantified by means of current molecular tools, its dynamics evaluated in sequential samples can be paralleled with clinical symptoms, it might induce highly serious diseases, particularly in immunocompromised patients, and it is a convenient target for inhibitors of viral DNA

polymerase. Although no extensive study has been done regarding the kinetics of viral replication in the human host, it can be speculated that the dynamics of quantitative viremia, at least to some extent, may predict HHV-6 disease risk, particularly in immunocompromised patients, as is the case for HCMV (122). However, one must keep in mind that many active HHV-6 infections are asymptomatic, even in the context of immune deficiency, and can revert to latency without any therapy. Moreover, if an active HHV-6 infection is suspected, it is essential to rule out the possibility of ciHHV-6. If the latter case is true, then virus reactivation may still have occurred and may be a potential source of disease. However, the patterns of transcription revealing active virus replication are not completely known, and the quantification of transcripts is not yet standardized, which makes the interpretation of virological findings ambiguous in many cases.

The development of extensive laboratory support, including the standardized quantification of viral nucleic acids and *in situ* detection of viral antigen expression in bodily tissues, is needed. Combined with the initiation of well-designed therapeutic trials, the improvement of laboratory approaches will open wide research perspectives on this pathogenic, albeit ubiquitous, human herpesvirus.

ACKNOWLEDGMENTS

We thank the editors and conference organizers with respect to the previously presented work mentioned in the introduction for giving us the opportunity to share our thoughts with the scientific community. We also express our gratitude to the HHV-6 Foundation, in particular to Dharam Ablashi, Kristin S. Loomis, and all the members of the scientific advisory board, for constant support, constructive comments, and stimulating discussions in the field of HHV-6 research.

This work was supported in part by the Université Pierre et Marie Curie (UPMC) and the Institut National de la Santé et de la Recherche Médicale (INSERM).

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