Human roseoloviruses include three different species, human herpesviruses 6A, 6B, and 7 (HHV-6A, HHV-6B, HHV-7), genetically related to human cytomegalovirus. They exhibit a wide cell tropism in vivo and, like other herpesviruses, induce a lifelong latent infection in humans. In about 1% of the general population, HHV-6 DNA is covalently integrated into the subtelomeric region of cell chromosomes (ciHHV-6). Many active infections, corresponding to primary infections, reactivations, or exogenous reinfections, are asymptomatic. They also may cause serious diseases, particularly in immunocompromised individuals, including hematopoietic stem-cell transplant (HSCT) and solid-organ transplant recipients, and acquired immunodeficiency syndrome (AIDS) patients. This opportunistic pathogenic role is formally established for HHV-6 infection and less clear for HHV-7. It mainly concerns the central-nervous system, bone marrow, lungs, gastrointestinal tract, skin, and liver. As the best example, HHV-6 causes both exanthema subitum, a benign disease associated with primary infection, and severe encephalitis associated with virus reactivations in HSCT recipients. Diagnosis using serologic and direct antigen-detection methods currently exhibits limitations. The most prominent technique is the quantification of viral DNA in blood, other body fluids, and organs by means of real-time polymerase-chain reaction (PCR). The antiviral compounds ganciclovir, foscarnet, and cidofovir are effective against active infections, but there is currently no consensus regarding the indications of treatment or specifics of drug administration. Numerous questions about HHV-6A, HHV-6B, HHV-7 are still pending, concerning in particular clinical impact and therapeutic options in immunocompromised patients.

INTRODUCTION

Human herpesviruses 6A, 6B, and 7 (HHV-6A, HHV-6B, HHV-7), are members of the family Herpesviridae, subfamily Betaherpesvirinae, and genus Roseolovirus. The three viruses are genetically close to each other and to cytomegalovirus (CMV), the type-species of human betaherpesviruses. Like other human herpesviruses, they are ubiquitous and establish a lifelong latent infection which originates further reactivations and reinfections. Early after the recent discovery of the three viruses, these properties, as well as the genetic relationship with CMV, have been a potent rationale for speculating on their potential pathogenicity in the immunocompromised population. The pathogenic role of HHV-6, this term collectively referring to HHV-6A and HHV-6B, is now ascertained in immunocompromised subjects whereas the clinical impact of HHV-7 infection appears less important in this domain. However, numerous questions are still pending, regarding diagnostic as well as therapeutic approaches targeted to these three viruses. Due to their strong similarities, the three viruses will be presented together throughout the chapter and specific details will be given for each of them within the different sections when needed.
VIROLOGY

HHV-6 was discovered in 1986 in patients with lymphoproliferative disorders (1). HHV-6 isolates were early stratified into two well-defined nonoverlapping groups that were first termed variants A and B and subsequently recognized as two distinct species, HHV-6A and HHV-6B (2, 3). HHV-7 was first isolated in 1990 from a healthy individual (4).

HHV-6A, HHV-6B, and HHV-7 share many common structural properties, as reviewed elsewhere (5–9). Virus particles contain a capsid of icosahedral symmetry surrounded by a tegument and an envelope; they are 150 to 200 nm in diameter. The genome is a linear double-stranded DNA, consisting of a unique (U) region flanked by identical terminal direct repeats (DRL and DRt); its approximate overall length is 160–170 kb (for HHV-6) and 145–153 kb (for HHV-7). DRL and DRt each contain two stretches of sequences related to the telomere repeat sequences (TRS) of vertebrate chromosomes. HHV-6 TRS include perfect repeats of the hexanucleotide GGGTTA, whereas HHV-7 TRS appear more heterogeneous. The overall number of protein-encoding open-reading frames (ORFs) ranges from 100 to 120, including a beta-herpesvirus-specific gene cluster, a set of genes unique to rosetroviruses, and few genes that neatly differ between HHV-6 and HHV-7, and/or between HHV-6A and HHV-6B. Overall amino-acid identity is about 50% between HHV-6 and HHV-7, while it is approximately 90% between HHV-6A and HHV-6B. Intraspecies genetic polymorphism appears wider for HHV-6A and HHV-6B than for HHV-7; in all cases, this polymorphism may provide useful markers for investigating both virus-transmission patterns and intraspecific recombination processes (10–13).

The intracellular replication cycle of rosetroviruses is assumed to follow the general scenario observed for other herpesviruses (8). Following attachment, virus entry into a cell occurs through a fusion process between viral envelope and cell membrane involving glycoprotein B (gB) and glycoprotein H (gH) functions. Viral DNA is released within the nucleus and viral genes are expressed in a temporarily ordered manner, starting with immediate-early (IE) genes and following with early (E) and late (L) ones. The replication of genome requires the synthesis of E proteins having an enzymatic activity dedicated to nucleotide metabolism and DNA synthesis, in particular the DNA polymerase. The packaging of progeny DNA into capsid precursors is followed by the acquisition of a final envelope in trans-Golgi network and release of mature virions by exocytosis.

PATHOGENESIS

HHV-6 infects a wide range of human cells, but preferentially replicates in activated CD4+ T lymphocytes in vitro; HHV-7 tropism is practically restricted to the latter cells (8). With respect to the cell components acting as virus receptors, note that HHV-6A uses CD46 while HHV-6B uses CD134 and HHV-7 uses CD4 (14–16). Generally speaking, HHV-6A exhibits a broader in vitro cell tropism than HHV-6B, and it is also the case for HHV-6 as a whole when compared with HHV-7. As for CMV, the in vivo host tissue range of HHV-6 and HHV-7 appears larger than that anticipated from in vitro studies. In vivo, HHV-6 infects central-nervous system (CNS) tissues, tonsils, salivary glands, kidney, liver, lymph nodes, endothelial cells, and monocytes/macrophages; HHV-7 is detected in lymphoid tissue, salivary glands, tonsils, liver, kidney, lungs, and skin. Following the entry of virus into the body through blood or respiratory pathways, the course of infection within the host is poorly known to date. Monocytes-macrophages and CD4+ T lymphocytes are assumed to be the sites of latent infection of HHV-6 and HHV-7, respectively (8). Whether the latent forms of virus correspond to sole DNA genomes organized as circular nuclear episomes, or to more complex structures permitting in-part transcription and even low-level replication, remains unknown.

The integration of HHV-6A and HHV-6B genome into human chromosomes (ciHHV-6) appears a unique feature among human herpesviruses, being even unreported for HHV-7 (17–19). This phenomenon has been described in 0.2% to 1% of the general population. The covalent linkage between viral and cellular DNA is observed within the subtelomeric region of chromosomes and is assumed to occur through a mechanism of homologous recombination between TRS of viral and cellular origin. Under this form, HHV-6 DNA can be transmitted to offspring through germ-line cells and to recipients through blood transfusion or organ/cell donation. In addition, ciHHV-6 might induce the production of viral transcripts, proteins, and even transmissible virosions, following reactivation (11, 20, 21).

The occurrence of a complete replication cycle of rosetroviruses has profound deleterious effects on host-cell functions and viability (5, 8). In addition, the discrete expression of certain viral genes might induce particular effects on the fine regulation of cell activity, such as the activation of transcription and transformation of cell phenotype. Of particular interest is the finding that some viral proteins encoded either by HHV-6 or HHV-7 have immunomodulatory functions. Those would
permit evasion of virus-specific immune response and modify the host-cell microenvironment in order to favor viral persistence. This is illustrated by the down-regulation of HLA class I expression by HHV-6 and HHV-7 U21 proteins and the synthesis of chemokine receptors encoded by U12 and U51 viral genes (22, 23). The immunomodulation capacity of roseoloviruses might also enhance the pathologic effects of other viral infections: HHV-6 is suspected to play a role as a cofactor of human immunodeficiency virus (HIV) in acquired immunodeficiency syndrome (AIDS), as well as in the activation of CMV and Epstein-Barr virus (EBV) (24, 25).

A specific immune response to HHV-6 and HHV-7 is observed in humans (8, 26). It is generated to diverse viral proteins, in particular some major antigens identified in immunoblot studies such as HHV-6 and HHV-7 U11 gene products. Cellular immunity is believed to play the major role in this response, as reflected by the deleterious effects of T-cell depletion on roseolovirus infection control in AIDS patients and transplant recipients. In the case of HHV-6, numerous T epitopes have been characterized, with the evidence of a significant cross-reactivity between HHV-6A and HHV-6B epitopes (27, 28).

### EPIDEMIOLOGY

The three roseoloviruses HHV-6A, HHV-6B, and HHV-7 are detected in all human populations around the world (8). Current serological assays do not permit discrimination of HHV-6A and HHV-6B infections, and HHV-7 seroprevalence studies are limited. As a whole, HHV-6 and HHV-7 infections are detected in more of 90% of the adult population in developed countries. HHV-6 infection is usually acquired very early in life, between six months and two years of age, starting from the moment at which protective maternal antibodies disappear (29). It is assumed that, in most countries, primary HHV-6B infection occurs first and is associated in many cases with clinical symptoms, whereas HHV-6A is acquired later and is asymptomatic. HHV-7 primary infection is believed to also occur in childhood but a bit later than HHV-6. Saliva is assumed to be the main vehicle for viral transmission, in agreement with the fact that the three viruses are frequently detected in saliva and salivary glands. Viral transmission through organ transplantation has been described infrequently and only in the case of HHV-6 (30). To date, blood transfusion and breast-feeding have not been found convincingly responsible for either HHV-6 or HHV-7 primary infections (31).

Congenital HHV-6 infection, i.e., the primary infection of embryo or fetus during pregnancy, has been found in about 1% of children, a frequency close to that observed for CMV, whereas congenital HHV-7 infection has not been detected to date. Cases of HHV-6 perinatal transmission leading to early primary infections have also been described. In contrast with CMV, congenital HHV-6 infection has a particular relationship with ciHHV-6 in pregnant women, which is not yet completely understood (32, 33). Although the frequency of ciHHV-6 is low in adults, this condition was indeed reported to be the predominant situation associated with a congenital HHV-6 infection resulting from the transplacental passage of virus. Prior to viral transmission, active HHV-6 infection of the mother would result from either an endogenous reactivation of latent virus (episomal genome or ciHHV-6) or from a putative exogenous reinfection. Note that in parallel, ciHHV-6 can be transmitted to offspring vertically through the germ line. In postnatal life, the transmission of organs or cells carrying ciHHV-6 through blood transfusion and transplantation does occur and raises relevant questions about its clinical impact on recipients.

### CLINICAL MANIFESTATIONS AND TREATMENT

#### Immunocompetent Hosts

HHV-6A, HHV-6B, and HHV-7 are the cause of human infections that are highly frequent and acquired during childhood, in most cases. Generally, these infections are asymptomatic or induce mild diseases (Table 1). HHV-6 primary infections cause acute febrile diseases associated with, to various degrees, fever, seizures, skin rash, and gastrointestinal and respiratory tract symptoms in young children from six months to three years of age (34). The most typical of these infant diseases is exanthema subitum (roseola infantum, sixth disease) (29, 35, 36). HHV-6B is the quasi-exclusive causative agent of this disease, HHV-6A primary infections being generally asymptomatic. Outcome is favorable in most cases. Primary infection with HHV-7 causes similar illnesses, but at a lower frequency than HHV-6B.

Infrequently, HHV-6 primary infection leads to more severe forms of disease such as hepatitis, including fulminant forms, thrombocytopenia, infectious-mononucleosis-like syndrome, hemophagocytic syndrome, gastroenteritis, colitis, myocarditis, and neurological complications (3). Meningoencephalitis represents the most severe manifes-
tation of CNS involvement during primary infection and its outcome remains uncertain, with a notable risk of death or neurologic sequelae. Recently, two fatal cases of encephalopathy following acute HHV-6 infection were reported in two children with underlying genetic mitochondrial disorders, which raises the question of predisposing conditions for these severe manifestations of primary infection (37). Severe forms of HHV-7 primary infections associated with neurologic symptoms have been reported but appear to be rare (38, 39). Cases of mesial temporal-lobe epilepsy have been putatively related to HHV-6B and the role of HHV-6 in febrile-status epilepticus has been discussed in children (29, 40–42). According to a recent report, 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 (43).

Following primary infection and establishment of latency, most reactivations and consequent reinfections are believed to be totally asymptomatic. Although they do not seem to have any impact on human health, these acute infections include the production of virus in body fluids, in particular blood and saliva, and they may play an important role in the transmission and maintenance of the three viruses among human populations.

**Immunocompromised Patients**

**General features**

Defects of cellular immunity appear to be the most prominent favoring factor of the emergence of active infections due to HHV-6A, HHV-6B, and HHV-7, and the induction of related clinical manifestations that may be severe and sometimes fatal. The quasi-universal distribution of these infections combined with their lifelong duration make any immunosuppressed individual at risk of developing such active infections. However, this concerning picture encompasses heterogeneous aspects that depend upon the variable clinical expression of immune deficiency and the complex interplay of roseoloviruses with the immune system and other pathogens, including CMV. The frequency and the location of organ infections appear to differ according to the underlying disease or condition, with distinct patterns

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<th>Stage of HHV-6 infection</th>
<th>Putative pathogenic effect</th>
<th>Associated diseasea</th>
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<tbody>
<tr>
<td>Congenital infection</td>
<td>Direct/indirect</td>
<td>CNS developmental defectsc</td>
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<td>Postnatal primary infection</td>
<td>Direct</td>
<td>Exanthema subitum (roseola infantum, sixth disease)</td>
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<td>Thrombocytopenia, infectious mononucleosis-like syndrome</td>
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<td>Hepatitis, gastroenteritis, colitis</td>
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<td>Pneumonitis</td>
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<td>Gastroenteritis, colitis</td>
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<td></td>
<td>Indirect</td>
<td>Drug-induced hypersensitivity syndrome</td>
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<td>Allograft rejectionc</td>
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<td></td>
<td></td>
<td>Thrombotic microangiopathyc</td>
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<tr>
<td></td>
<td></td>
<td>Higher incidence and severity of infections with CMV, fungi, and other opportunistic pathogensc</td>
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<tr>
<td>Chronic infection</td>
<td>Indirect</td>
<td>Multiple sclerosisc</td>
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<td>Acceleration of evolution to AIDS in HIV-positive individualsc</td>
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-CNS, central nervous system; CMV, human cytomegalovirus; AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus.

Indirect effect is mainly related to putative immune dysfunction.

To be confirmed.
affecting AIDS patients, hematopoietic stem cell transplant (HSCT) or solid-organ transplant (SOT) recipients. Disease spectrum may involve many organ systems: the CNS, including retina, is a major target in agreement with the known neurotropism of human roseoloviruses; bone marrow, lungs, gastrointestinal tract, and liver are also often concerned (Table 1). The spectrum of clinical manifestations is wide regarding the intensity of symptoms as well as their specific location. However, as a whole, the clinical impact of HHV-7 infections appears moderate in immunocompromised subjects when compared with HHV-6 effects. As the seroprevalence of HHV-6 and HHV-7 infections is very high in the adult population, viral reactivations are thought to constitute the vast majority of active infections among adult patients. In contrast, primary infections are more common in pediatric patients than in adults, and that might affect the clinical expression of active infections in immunocompromised pediatric patients. Lastly, the clinical impact of ciHHV-6 in transplant patients remains to be evaluated. Viral reactivation from integrated viral DNA might be enhanced due to immunosuppression context, and thus have deleterious effects either directly or indirectly on the outcome of the transplantation process. That urgently requires further investigations on both aspects of the problem—ciHHV-6 recipients and recipients of ciHHV-6 transplanted organs—and raises the question of the determination of ciHHV-6 status for recipients and donors in the future (19).

HSCT recipients
As HSCT recipients are concerned, some of the clinical symptoms associated with HHV-6 reactivations may be considered nonspecific, such as fever, rash, and transient decreased number of circulating blood cells belonging to granulocyte/macrophage, erythroid, and megakaryocytic lineages (Table 1). In contrast, subacute limbic encephalitis is now recognized as a typical opportunistic disease due to HHV-6 reactivation in such patients (44, 45). These patients, particularly those receiving allogeneic transplant, are at high risk of developing a reactivation within the first four weeks after cell transfer. However, encephalitis only develops in a small proportion of patients experiencing HHV-6 reactivation. The majority of cases are due to HHV-6B. The direct effect of HHV-6 on the CNS was confirmed in a recent study that demonstrated a strong correlation between HHV-6 reactivation and CNS dysfunction as measured by delirium and neurocognitive decline in HSCT patients (46). The other major complication associated with HHV-6 reactivation in HSCT patients is bone-marrow suppression, which may evolve to secondary graft failure (47). This is supported by numerous clinical reports and may be related to the latent infection of bone marrow progenitors in the general population (48, 49). Other disease associations have been reported for HHV-6 reactivation: graft-versus-host disease, thrombotic microangiopathy, CMV reactivation, pneumonitis, and gastrointestinal disease (47). In those cases, the causative relationship is less clear due to conflicting results, complexity of HHV-6 interactions with host immune system, and possible confusion with the effects of other concomitant pathogens, such as CMV.

Reactivation of HHV-7 is commonly observed in HSCT recipients, including pediatric patients, and usually occurs earlier than for HHV-6, within the first weeks after transplant; in contrast, clinical diseases directly due to HHV-7 seem very rare in these patients (50–52).

SOT recipients
In SOT patients, primary HHV-6 infections are very rare, except in pediatric transplant recipients. HHV-6 reactivations are frequently detected in adults, but occur at very different rates according to the characteristics of organ transplant, nature of immunosuppressive therapy, and administration of a prophylactic anti-CMV treatment, which is assumed to be also active against HHV-6 (5, 44, 53, 54). HHV-6 reactivation generally occurs at two to four weeks after transplantation and HHV-6B is the main species detected. These infections remain asymptomatic in many cases. Nevertheless, concomitant febrile episodes and other nonspecific symptoms, such as leukopenia and thrombocytopenia, have been often observed (55). Specific severe diseases have been also reported in SOT patients, but the causative relationship with HHV-6 does not appear to be as convincing as in HSCT patients. In renal-transplant recipients, the spectrum of clinical manifestations seems extremely limited, whereas hepatitis, pneumonitis, bone-marrow suppression, and encephalitis have been reported for liver, lung, and heart-transplant patients (53, 56). In this context, a synergistic pathogenic role of CMV has been suggested. The role of HHV-6 in graft rejection remains a debated question, as is the facilitation of superinfections with fungi and other opportunistic pathogens (55, 57).

The detection of HHV-7 DNA in the blood from SOT recipients is very frequent, affecting up to 40% of patients, as it is also observed in studies involving unselected human populations (13, 58–60). Although HHV-7 reactivation is commonly observed within the first two to four weeks after SOT, its magnitude and...
duration are most often limited (58). The association of HHV-7 reactivation with clinical manifestations, such as fever and thrombocytopenia, is therefore debated and often confused with the occurrence of concomitant CMV infection and disease, with a strong suspicion of positive interaction between both viruses (61, 62).

**HIV-positive patients**

In HIV-positive patients, the clinical impact of HHV-6 reactivations was considered important before the development of antiretroviral therapy, similarly with the prominent pathogenic role observed for CMV in this context. An increased frequency of active infections was observed in late stages of the AIDS course in the context of a deep immunodeficiency, but a specific deleterious role of HHV-6 reactivations was questioned for earlier stages (63, 64). As a preliminary conclusion, the capability of HHV-6 to accelerate the progression to AIDS remains debated, while its opportunistic role among AIDS-associated infections was convincingly demonstrated by the report of cases of encephalitis, pneumonitis, and retinitis (65–68).

**Patients Presenting Immune Dysfunction**

Both experimental and clinical considerations led to evoke the pathogenic role of roseoloviruses in diverse diseases related to an abnormal activity of the immune system. Indeed, these viruses have the capability to modulate the host immune system regarding both innate and adaptive immune responses (69). Various syndromes that share many clinical and biological features with those of frank immunosuppression situations have been associated with roseolovirus infections (Table 1).

Drug-induced hypersensitivity syndrome, also known as drug rash with eosinophilia and systemic symptoms (DRESS), is constituted of severe adverse drug reactions, including various degrees of skin rash, fever, lymph-node enlargement, liver dysfunction, and abnormalities of blood leukocytes. This syndrome appears to be often related to active HHV-6 infection in such a way that, in Japan, HHV-6 active infection is part of the criteria used for its diagnosis (70–73). As in the case of EBV, another human herpesvirus suspected to have a role in this syndrome, the starting event would be the triggering of HHV-6 multiplication by the responsible drug, resulting in immune activation and inflated antiviral T-cell response generating the disease (73, 74). Accordingly, drugs inducing the syndrome, such as amoxicillin and sodium valproate, have been experimentally shown to directly stimulate HHV-6 replication (7, 75, 76). As far as other skin diseases are concerned, pityriasis rosea is an acute self-limiting exanthematous illness that has been related to the reactivation of HHV-6 and/or HHV-7 and might lead to prolonged forms also associated with persistent active viral infection (77–79). Although the data remain conflicting in this domain, this disease might be the clinical expression of an altered immune response to roseolovirus reactivation.

HHV-6 might act as a possible trigger for multiple sclerosis (MS), an autoimmune demyelinating disease of the CNS. Although this has been debated for a long time, HHV-6 appears as a valuable candidate in the long list of potential infectious triggers on the basis of immunological, virological, and experimental data (40, 80, 81). That comprises the isolation of this virus from diseased CNS tissues in MS patients, study of HHV-6 antibody reactivity in their serum and cerebrospinal fluid (CSF), and in situ detection of HHV-6 transcripts (80, 82–86). Hashimoto’s thyroiditis is another autoimmune disease in which HHV-6A infection is suspected to act as an environmental trigger (87, 88).

Chronic-fatigue syndrome is a chronic disease characterized by a major functional impairment and underlying biological abnormalities, which include markers of chronic immune activation as well as neuroendocrine dysfunction (89). Many reports have suggested the possible causative role of HHV-6 in this syndrome as supported by an increased rate of viral-reactivation events (90–92).

**Therapeutic Options**

Three drugs, already used against CMV infections, have been shown to be active against HHV-6A, HHV-6B, and HHV-7 infections in vitro: ganciclovir, a nucleoside analogue; foscarnet, a pyrophosphate analogue; and cidofovir, an acyclic nucleoside phosphonate (5, 93–97). The mechanism of their antiviral activity is similar for CMV, HHV-6, and HHV-7. The common final target is the viral DNA polymerase that is specifically inhibited by the triphosphorylated form of ganciclovir, the diphosphorylated form of cidofovir, and directly by foscarnet, which does not require any chemical modification for antiviral activity. The first phosphorylation step of ganciclovir is catalyzed by a phosphotransferase, encoded by the HHV-6 and HHV-7 U69 genes, while the further phosphorylation steps of ganciclovir and the two phosphorylation steps of cidofovir are dependent upon the activity of cellular kinases. HHV-6 and HHV-7 do not express any thymidine kinase, the enzyme that performs the first phosphorylation step of acyclovir, penciclovir, and brivudin, which explains in part the low activity of these drugs against roseoloviruses.
In agreement with the mechanism of antiviral activity, acquired virus resistance to ganciclovir, foscarnet, and cidofovir has been related to mutations of phosphotransferase and DNA-polymerase genes (U69 and U38 genes, respectively) in the case of HHV-6 (98–102). Interestingly, some of these mutations may be selected in the context of antiviral treatments given against other herpesviruses, as illustrated by the report of HHV-6 resistance to ganciclovir following the prolonged treatment of CMV infection with this drug (98). In addition, despite their selectivity with respect to viral enzymes, efficient anti-roseolovirus drugs exhibit a certain degree of cell toxicity. That may restrict their use, particularly in immunocompromised patients, and relates to bone marrow in the case of ganciclovir and renal toxicity in the case of foscarnet and cidofovir. Note that brincidofovir, also known as CMX001, is an orally administered lipid-ester derivative of cidofovir that is active against numerous DNA viruses, including HHV-6 and HHV-7, is detected at significant levels in CSF from treated patients, and is less toxic for the kidney than cidofovir (95, 103, 104). As a whole, the usefulness of these drugs against diseases thought to be caused by roseoloviruses has been reported in humans, but only in the context of uncontrolled studies (6). Therefore, none of these drugs is officially approved for use against HHV-6 or HHV-7 infections in vivo.

Other potentially efficient nucleoside or nucleotide analogues are currently in preclinical development or in early clinical trials (94, 95, 105, 106). Artesunate, a derivative of artemisinin used for the treatment of malaria, has been shown to inhibit the replication of CMV and HHV-6 and was used to treat CMV infections in vivo as a compassionate use (107, 108). Recently, the use of complementary immunotherapy, particularly in the context of HSCT, has been considered using polyclonal cytotoxic T lymphocytes targeted to several opportunistic viruses, including HHV-6 (28, 94). The concept of an adoptive therapy based on T-cells is very attractive, due to the possibility of circumventing drug cytotoxicity and resistance, but requires a validation in ongoing clinical trials.

**Treatment Indications and Monitoring**

In parallel to any antiviral drug administration, a key strategy for the control of active roseolovirus infections is the reversal of immunosuppression when this favoring factor can be down modulated. Thus, reducing the dose of immunosuppressive drugs has to be considered as the first approach when this option is available (54, 109).

To date, the question of antiviral-therapy administration has been raised for HHV-6 infections. In this domain, the experience developed in anti-CMV treatments might help to make a choice between prophylactic, preemptive, and curative approaches (9). The available drugs are efficient against active infections only, these consisting of either primary infections (mainly in young children) or reactivations (in most adults). Prophylactic treatment has the advantage of conferring an overall protection against any active infection. However, it would require the treatment of numerous individuals, in particular virtually all immunocompromised patients, induce high costs, and expose patients to side effects, as well as to possibly select for resistant viruses. On the contrary, curative therapy would be initiated only once the diagnosis of HHV-6-associated disease has been made, which would concern much less people but expose them to the risk of therapeutic failure due to late intervention. The median solution of preemptive therapy, in which the detection of a significant viral replication is the starting signal for therapy prior to any declared disease, has proved to be efficient and cost-effective in the case of CMV infections (110). However, preemptive therapy would require the precise knowledge of HHV-6-replication dynamics and relevant prediction of disease occurrence, which, to be honest, is not yet achieved. Many active HHV-6 infections are spontaneously controlled in immunocompromised patients and we do not know the reason why. In addition, the validation of therapeutic options requires randomized controlled trials, which are missing to date (94). Therefore, it seems premature to propose either prophylactic or preemptive therapies in order to prevent clinical manifestations related to HHV-6 infections.

Provisionally, some additive criteria may be proposed for initiating therapy against HHV-6 active infections: (i) significantly high viral load in the absence of ciHHV-6; (ii) immunosuppression context; (iii) clinical symptoms correlating to concomitant virus replication based on a relevant pathophysiological context; and (iv) absence of any other causative agents, in particular CMV. Indeed, HHV-6-associated encephalitis in HSCT patients fulfils most of these criteria and appears a potential priority target for antiviral therapy. Accordingly, the International Herpesvirus Management Forum and the American Society of Transplantation, which did not recommend antiviral prophylaxis for HHV-6 infection, promoted the initiation of antiviral therapy in case of HHV-6 encephalitis, intravenous ganciclovir and foscarnet being proposed as first-line treatments for approximately three to four weeks (54, 55, 59). No other
recommendation has been internationally approved for the therapy of active HHV-6 infections while numerous questions are pending. The severe neurological manifestations associated with primary infection in children, particularly in the context of primary immunodeficiency diseases, might be another priority indication, but the data supporting this proposal need to be strengthened.

The effectiveness of antiviral therapy has to be monitored on both the concomitant regression of clinical symptoms and decrease of viral load, as in the case of anti-CMV treatments (110). A diverging evolution of these parameters would make one suspect a causative agent other than the targeted roseolovirus. A therapeutic failure in spite of the prolonged administration of an efficient antiviral drug may reveal the emergence of a resistant virus and lead to a drug susceptibility test being performed, currently based on genetic analysis, this test being not widely available in most medical centers (111). It is acknowledged that the resistance of roseoloviruses to antiviral drugs is not currently a crucial question as it is for CMV (110). However, the risk of resistance selected through a previous exposure to drugs in the context of other concomitant herpesvirus infections, notably CMV ones, must be kept in mind (98).

LABORATORY TESTING OPTIONS AND APPLICATIONS

Specimen Types and Processing

Two complementary approaches can be utilized (112). Direct diagnosis is based on the detection and characterization of whole-virus particles or their internal specific components, the most convenient targets currently being nucleic acids. These components are detected in either cell-free virions or infected cells that contain not only the components of released viral particles, but also transcripts and additional virus-encoded proteins participating in the intracellular-replication cycle. Indirect diagnosis, also known as serology or serological diagnosis, is based on the detection and characterization of virus-specific antibodies in a body fluid, typically serum, using reference viral antigens.

These two diagnostic approaches offer distinct advantages and disadvantages in terms of sensitivity, specificity, time requirements, and result interpretation (Table 2). They each provide different information and the combination of both is rarely necessary, provided the implementation of the selected method has been adequately decided according to the question in debate.

Patient specimens also have to be appropriate for the selected diagnostic process. Whole blood, plasma, and serum are frequently used and readily accessible. CSF is needed for the diagnosis of CNS infections, whereas bronchoalveolar lavages permit the diagnosis of lung infections. Generally speaking, any cellular fraction obtained from a body fluid, cell smear, or tissue biopsy can be tested for the presence of viral components by means of direct diagnostic assays. Saliva samples have the advantages of being readily obtained, easily stored, and loaded with virions (and/or viral DNA), infected cells, and antibodies (113). Their relevance for diagnosis, however, needs clarification. Conversely, the frequent

<table>
<thead>
<tr>
<th>General approach</th>
<th>Technique</th>
<th>Advantages and applications</th>
<th>Disadvantages and limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serology (IFA, EIA)</td>
<td>Detection and avidity assays for IgG and IgM</td>
<td>Easy collection and storage of serum samples</td>
<td>Interpretation in case of virus reactivation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Readily accessible techniques</td>
<td>No discrimination HHV-6A/HHV-7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diagnosis of primary infection</td>
<td>Delayed/altered response if immunosuppression</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serorevaluation studies</td>
<td>Cross-reactivity with other beta-herpesviruses</td>
</tr>
<tr>
<td>Direct diagnosis</td>
<td>Virus isolation in cell culture</td>
<td>Reference method in virology</td>
<td>Labor-intensive method</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Evidence of infectious virus</td>
<td>High cost</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Further investigations on virus strains</td>
<td>Limited sensitivity</td>
</tr>
<tr>
<td></td>
<td>Antigen detection</td>
<td>Evidence for virus gene expression</td>
<td>Limited availability of reference antibodies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Discrimination HHV-6A/HHV-7</td>
<td>No distinction between active infection, latency, and ciHHV-6</td>
</tr>
<tr>
<td></td>
<td>Qualitative viral DNA PCR</td>
<td>High sensitivity and specificity</td>
<td>Need for international standardization</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Discrimination HHV-6A/HHV-7</td>
<td>Need for specific thresholds in active infections</td>
</tr>
<tr>
<td></td>
<td>Quantitative viral DNA real-time PCR</td>
<td>Longitudinal follow-up studies</td>
<td>and ciHHV-6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Comparison of viral loads in blood vs organs</td>
<td>Limited sensitivity (to be evaluated)</td>
</tr>
<tr>
<td></td>
<td>Detection of viral transcripts by RT-PCR</td>
<td>Distinction between active and latent infections</td>
<td>Need for standardization</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Precise measurement of nucleic acid amounts</td>
<td>Limited sensitivity (to be evaluated)</td>
</tr>
<tr>
<td></td>
<td>Droplet digital PCR</td>
<td>Identification of ciHHV-6</td>
<td>Adaptation to clinical specimen diversity</td>
</tr>
</tbody>
</table>

*IFL, immunofluorescence assay; EIA, enzyme immunoassay; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-polymerase chain reaction; ciHHV-6, chromosomally integrated human herpesvirus 6.*
Serology
The serologic methods dedicated to roseolovirus infections are mainly founded on indirect immunofluorescent antibody assays (IFA). In such assays, cells are infected with the target reference virus, fixed onto glass slides, and then submitted to the reaction with the serum to test. The readout with an optical microscope relies on both the number of fluorescent foci and intracellular distribution of staining, taking also into consideration nonspecific background-fluorescent signals. Neutralizing antibodies are considered more specific and correlated with protective immunity, but their identification, based on cell-culture assays, is cumbersome and expensive (114, 115). Enzyme immunoassays (EIA), which use either crude infected-cell lysates or purified virus as antigens, have been developed to a lesser extent (116, 117). Despite the obvious convenience of EIA, their specificity has been repeatedly questioned. In the future, the use of synthetic peptides as antigens might bring significant progress, especially if immunoblot assays, considered much more specific than EIA, and antibody-avidity tests are developed in parallel (118–120).

Antibodies remain stable markers even after prolonged storage of samples. Therefore, serologic assays provide reproducible results, particularly convenient for retrospective studies. However, the interpretation of these results may be equivocal in many aspects. Roseolovirus seropositivity is highly prevalent and poorly discriminant in the adult population. The dynamics of IgG responses and presence of IgM antibodies are not totally specific for acute primary infections since they may be also observed during viral reactivation. Antibody cross-reactivity has been reported not only between the three human roseoloviruses, but also between them and CMV (121–124). The serologic assays targeting HHV-6 antibodies that are currently available cannot differentiate HHV-6A from HHV-6B infections. Lastly, the serologic reactivity patterns may be atypical in case of immune suppression or ciHHV-6. Clearly, our knowledge of humoral-immune response against HHV-6 and HHV-7 at different stages of infection (primary production, latency, and ciHHV-6) must be improved to permit the development of better serological assays. These limitations tend to restrict the current indications of serology to the diagnosis of primary infection, identification of uninfected subjects, and studies of seroprevalence. Consequently, serology has a limited usefulness in the management of immunocompromised patient infections during which antibody response may be poor or absent (112).

Viral Culture and Antigen Detection
The isolation of HHV-6A, HHV-6B, and HHV-7 in cell cultures is a reference method that permitted the discovery of these viruses and unambiguously demonstrates the presence of infectious viral particles in a sample (1, 4, 35). However, this method is poorly sensitive, time-consuming, and expensive. It cannot be used for routine diagnosis and is not available in most medical centers. All HHV-6 isolates theoretically grow in peripheral-blood mononuclear cells (PBMCs) or cord-blood lymphocytes leading to a cytopathic effect, made of enlarged and refractive cells, which might be missing in some cases. The isolation on other cell types, such as fibroblasts, and the growth adaptation to cell lines are possible, in particular for HHV-6A, but even more difficult than culture on primary permissive cells (125, 126).

The detection and approximate quantification of viral antigens in PBMCs and tissue biopsies enables the observation of viral proteins expressed at different stages of infection (127, 128). This is particularly useful for locating active viral infections inside of tissue lesions using immunohistochemistry techniques. This approach can be combined with the detection of viral DNA by in situ hybridization (129, 130). These methods permit the investigation of intracellular distribution of antigens or viral nucleic acids and, if optimized, might be of potential value in differentiating HHV-6 infection from graft-versus-host disease. However, the panel of available reference antibodies that can be used in such assays is limited, there is a risk of cross-reactivity between the different betaherpesviruses, and the sensitivity of detection is considered low with current reagents. For those reasons, antigen-detection studies and histological investigations are currently dedicated to research objectives rather than diagnostic procedures.

Nucleic Acid Detection and Quantification
The detection and quantification of viral nucleic acids have become the gold standard of diagnostic procedures applied to HHV-6A, HHV-6B, and HHV-7. By means of numerous assays based on real-time PCR, viral DNA can be detected and reproducibly quantified in a broad range of clinical specimens, including whole blood, CSF, and any other bodily fluid or tissue (131–133). The methods are financially accessible, quick, safe, and currently widespread. Novel developments have largely solved previous problems, such as the nonspecific inhibition of
DNA amplification, carryover inside the labs, and limited range of signal linearity for quantification. In addition, these approaches also readily differentiate the two species of HHV-6, even in cases of mixed infection (132). Multiplex-PCR assays have been set up to detect and/or quantify roseoloviruses and other human herpesviruses concomitantly (136, 137). However, there is an obvious need for standardization of the various molecular assays under usage in order to permit the unambiguous comparison and interpretation of results obtained in different laboratories; in that context, the introduction of reference international standards for HHV-6A, HHV-6B, and HHV-7 would constitute a significant advance, as observed recently for CMV diagnosis (110).

The detection and quantification of viral transcripts is a valuable complementary approach, primarily used for the research purpose of recognizing the different steps of the virus cycle, rather than for clinical diagnostics. However, the detection of late-gene transcripts would help to identify productive infections, whereas the finding of a remote detectable amount of latency-specific transcripts (as exemplified by HHV-6 U94 gene transcripts) would reveal a predominantly latent phase of infection (138–140). In many assays, the detection of transcripts is facilitated by targeting spliced mRNAs, the amplimers obtained from transcripts 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. Temporal analysis of HHV-7 viral transcripts has been published, but the application for diagnosis have been limited to date (141).

**Molecular Analysis of Nucleic Acids**

A precise molecular characterization of viral DNA and transcripts has been warranted by recent questions about the epidemiology and physiology of HHV-6 and HHV-7 infections: clear distinction between HHV-6A and HHV-6B species among HHV-6 infections, mechanism of transplacentally acquired congenital infection, viral reactivation from ciHHV-6, occurrence of exogenous reinfections, and concomitant infections with HHV-6A and HHV-6B. Molecular analysis also allows the genetic detection of resistance to antivirals when targeting specific mutations known to confer a decreased susceptibility to drugs (99, 142).

The general approach is the combination of gene amplification, nucleotide sequencing, and phylogenetic study of selected loci of viral DNA. Moreover, droplet-digital PCR and next-generation sequencing, which constitute recent developments of molecular techniques, offer novel opportunities for investigations in this complex domain (143–145).

Although knowing the causative HHV-6 species precisely has no impact on diagnosis or management at present, it permits the identification of infections due to HHV-6A, HHV-6B, or a mixture of both when a diagnosis of HHV-6 infection has been made (9, 146). This information will prove valuable in defining the spectrum of diseases associated with each of the viruses, and in the event virus-specific therapeutic options become available. Remember that this differentiation step is still not possible using conventional serologic assays. As a whole, HHV-6B is by far the most frequently detected species in peripheral blood, saliva, and CSF, both in asymptomatic infections and in diseases potentially associated with HHV-6 (5, 132, 147, 148). 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. 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 a mystery to date.

**DIAGNOSTIC ALGORITHM, RESULT INTERPRETATION, AND CLINICAL RELEVANCE**

**Algorithm for Diagnosis of Active HHV-6 Infections**

Many questions are pending regarding the pathogenicity of roseoloviruses in humans and investigations that should be developed to improve our knowledge in this area. As described above, therapeutic options appear limited but can be used provided that the indications of therapy are clarified and strongly sustained by proper virological findings. In that context, active HHV-6 infections and severe associated diseases may be considered a priority for diagnosis for many reasons. The pathogenic role of HHV-6A and HHV-6B is certain, in particular among immunocompromised patients, although its manifestations have yet to be completely explored. Active HHV-6 replication can be confidently diagnosed, quantified, and sequentially investigated by means of current molecular tools, which are functional albeit imperfect. The kinetics of viral replication in the human host has not been accurately studied, but it can be speculated that the dynamics of quantitative viremia, at least partially, may predict HHV-6 disease risk, particularly in immunocompromised patients as for CMV (110). Active HHV-6 infections are convenient targets for diagnosis, and nucleic acid-based detection methods offer a valuable approach for investigations.
for the utilization of viral DNA-polymerase inhibitors. On the other hand, the results of virological investigations have to be considered with caution. If an active HHV-6 infection is suspected, ciHHV-6 has to be ruled out. Conversely, if the hypothesis of ciHHV-6 is true, viral reactivation may still occur and act as a potential source of disease. Many active HHV-6 infections are asymptomatic, even in the context of immune deficiency, and can revert to latency without any therapy. In that context, an algorithm can be proposed for the diagnosis, monitoring, and therapy of severe HHV-6 infections (Fig. 1). This emphasizes the need for the development and standardization of laboratory techniques dedicated to these objectives.

**Primary Infection**

Primary infections with HHV-6 or HHV-7 can be identified by the detection of specific IgM antibodies and/or a seroconversion regarding specific IgG response. Note that, in any situation of immunodeficiency, humoral-immune response may be delayed and/or absent. The presence of the maternal IgG antibodies also may interfere with serological diagnosis during the first months of life. In addition, current serological assays do not permit differentiation of HHV-6A and HHV-6B primary infections. Alternatively, the detection of virus DNA in whole blood, PBMCs, plasma, or saliva by means of PCR in the absence of any previously known infection by HHV-6 or HHV-7 replaces the classical isolation of virus in cell culture and constitutes a proof of primary infection (33, 149, 150). However, as serologic tests are not highly reliable in immunocompromised patients, the notion of a previous roseolovirus infection may be difficult to establish and the predictive value for primary infection of a positive PCR result in any sample type is not known. When seizures or encephalitis symptoms are associated with cutaneous rash, the detection of viral DNA in CSF permits the confirmation of viral replication in CNS. Other biological samples may be analyzed according to the nature and location of clinical symptoms.

**FIGURE 1** Proposal of algorithm for the diagnosis and therapy monitoring of HHV-6-associated disease in an immunocompromised patient. It must be recalled that some of the virological tests mentioned in this algorithm are not widely available in medical centers and that the predictive value of positive results for the diagnosis of infection or disease is not formally established for many of the indicated sample types.

<table>
<thead>
<tr>
<th>Immunocompromised patient with fever, rash, and/or specific organ-related symptoms</th>
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<td><strong>Suspect HHV-6 associated disease</strong></td>
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<td><strong>Check for active HHV-6 infection</strong></td>
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<td><strong>Check for HHV-6 organ involvement</strong></td>
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<td><strong>Exclude or confirm ciHHV-6</strong></td>
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<td><strong>Consider HHV-6 therapy</strong></td>
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<td><strong>Monitor HHV-6 therapy</strong></td>
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<td><strong>Consider HHV-6 resistance to antivirals</strong></td>
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Congenital infection, corresponding to the transplacental transmission of a replicative virus from mother to child, can be diagnosed at birth by the detection of specific IgM antibodies and viral DNA in cord or peripheral blood. However, the complex imbrication between the congenital HHV-6 infection of babies and frequent ciHHV-6 status of their mothers may require the use of additional markers, such as the precise quantification of viral DNA, study of late transcripts, and detection of HHV-6 DNA in hair-follicle samples according to a diagnostic strategy that yet needs to be established (32, 33).

Latent Infection
Latent infection corresponds to the widespread chronic infection as classically described for all human herpesvirus, and also to ciHHV-6, which is only present in a minority of the general population and deserves a specific consideration (see below). The clinical syndromes possibly related to latent roseolovirus infection in immunocompromised populations are currently unknown. Seropositivity appears to be a convenient marker for latent infection, although the limitations of serologic assays have been already mentioned. The detection of virus in blood is infrequent, and, when detected, virus load is low. In a recent study concerning 200 healthy blood donors, viral DNA was detected in whole blood in 8%, 0%, and 51% of studied subjects for HHV-6B, HHV-6A, and HHV-7, respectively. One subject had ciHHV-6. In the other subjects who had a detectable DNAemia, median loads were 81 and 129 copies per million PBMCs in HHV-6B and HHV-7, respectively (151). The detection and quantification of HHV-6 U94 gene transcripts have been suggested to provide markers for latency status and, in some cases, ciHHV-6 (152). However, the major hindrance for the detection of direct viral markers during latency is, by definition, their intrinsically low level of expression.

Reactivations
In the context of reactivations, serological approaches are useless and a detectable viremia is considered the hallmark of a systemic active infection (7, 58, 153). For many authors, whole blood is now considered a valuable support for such detection by means of real-time PCR (135, 154). Alternatively, PBMCs can be used as well as plasma, which is easier to handle and store than whole blood and PBMCs. However, the use of plasma raises questions about the origin of viral DNA detected in this compartment: this DNA corresponds either to real virus production from lymphoid tissue, the measurement of which is relevant in terms of virus replication, or release from incidentally lysed circulating cells, which may increase plasma load artificially (155).

Due to high sensitivity of real-time PCR and prevalence of latent HHV-6 and HHV-7 infections in adults, the qualitative detection of viral DNA in blood is frequently positive, even in healthy individuals, as already mentioned. Therefore, to be correctly interpreted, DNAemia has to be precisely computed. To date, no threshold formally separates latent and active infections. As a first approximation, the value of 1,000 genome-equivalent copies per mL of whole blood delineates a border area between the two stages of HHV-6 infection. This value broadly corresponds to 1,000 DNA copies per million PBMCs when the white-blood-cell count is within a normal range (50, 156, 157). Viral loads corresponding to active infections usually range from 1,000 up to several hundreds of thousands of copies per mL of whole blood. The most-elevated values may be confused with DNAemia values resulting from ciHHV-6. In this case, HHV-6 DNA is present in every nucleated cell, particularly in cells from hair follicles, nails, CNS, and peripheral blood. In ciHHV-6 subjects, HHV-6 DNAemia in whole blood is usually over one million copies per mL, far beyond the values observed in most active infections (135, 158). However, when white-blood-cell count is decreased, as can be seen in HSCT recipients, the values of absolute viral load may be ambiguous. In this case, the expression of results as copies per cell may help to differentiate ciHHV-6 from most active infections in which average viral load is usually lower than one copy per cell (159). Droplet-digital PCR was also recently shown valuable for the identification of ciHHV-6 by precisely determining the ratio of HHV-6 to cellular DNA without the use of a standard curve (144, 153). Our knowledge of quantitative DNAemia in active HHV-7 infections is far more limited than for HHV-6 and further complicated by previous findings indicating that HHV-7 loads were similar or higher in healthy control subjects than in HSCT and HIV-positive patients (50, 160).

When an active infection appears restricted to a specific body compartment (CNS, gastrointestinal tract, lung, liver, skin), viral loads in whole blood and in a sample from this compartment, when accessible and when validated tests are available, can be compared with each other in order to estimate the respective parts of blood input and in situ virus multiplication. In this situation, the value of viremia does not need to be significantly elevated for establishing the diagnosis of active infection. Due to the sensitivity and specificity of current PCR techniques, the detection of roseolovirus DNA in
CSF is thought to be sufficient per se for the diagnosis of an active infection of CNS, although this assumption appears more controversial in the case of HHV-7 (39, 161, 162). Restricted local viral reactivations in CNS seem possible, as suggested in some cases of HHV-6 encephalitis after HSCT (103, 163). In this context, the quantification of HHV-6 DNA in CSF, instead of its simple qualitative detection, may be worthy, particularly in case of longitudinal follow-up. Note that, in ciHHV-6 patients, unusually high HHV-6 DNA loads in body fluids may be interpreted falsely as an acute infection in these body compartments, as described for CSF samples (164).

The detection and/or quantification of transcripts specific to a productive viral cycle, for instance late-gene transcripts, would be helpful to characterize active infections. However, several criteria are required to implement this strategy: value of target messenger RNAs as specific markers of active infection, sufficient sensitivity of the approach, standardization of RNA-quantification procedures, and definition of precise thresholds for interpretation. To date these criteria have not been fulfilled. The same comment could be made regarding the detection of specific virus-encoded proteins, by means of their antigenic properties or using mass-spectrometry techniques (165).

Note that the active infection resulting from an exogenous reinfection with another strain of the same roseolovirus species appears possible, but would be very difficult to differentiate from a commonplace endogenous reactivation. For that purpose, molecular-typing procedures, including deep sequencing of viral subpopulations, should be implemented.

**Chromosomal Integration of HHV-6 DNA**

The existence of ciHHV-6 notably interferes with the diagnosis of active HHV-6 infection in immunocompromised patients, leading in some cases to falsely positive conclusions and unnecessary treatments (166). Conversely, viral reactivation from ciHHV-6 has been reported and has to be considered in terms of clinical impact since it is suspected to be implicated in congenital infection (11, 21). Therefore, ciHHV-6 might modify the frequency of adverse effects and the overall prognosis of transplantation and transfusion in patients receiving ciHHV-6 blood cells or organs (18). Further research is needed to clarify those points and provide clear responses to persons carrying ciHHV-6, when detected.

Fluorescence in situ hybridization on EBV-transformed B-lymphocytes was originally used to identify ciHHV-6, but this assay is complex, cumbersome, and expensive (167). The finding of a viral load equal or higher than one DNA copy per cell, in whole blood and, to a lesser extent, in other body fluids such as CSF, suggests the presence of ciHHV-6. It should encourage searching for the presence of HHV-6 DNA in hair follicles or nails, which is a readily accessible signature of ciHHV-6 (158, 168). Additional indirect evidence is provided by the persistence of unchanged high viral load over time, even after initiation of an antiviral therapy. Note that the production of HHV-6-specific serum antibodies is altered in certain ciHHV-6 individuals, presumably through a phenomenon of immune tolerance, which constitutes an additional challenge for diagnosis (18).

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