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Editorial overview: Roseoloviruses: Stopping to smell the roses — the Roseoloviruses have come of age as human pathogens

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Dr Laurie T Krug is an Assistant Professor at Stony Brook University. She began her research career as a graduate student in Dr Philip Pellett's laboratory studying the human herpesvirus 6B U94 gene and Roseolovirus origin-binding proteins. While a postdoctoral fellow with Drs Margaret Offermann and Samuel Speck at Emory University she studied the molecular biology, immunobiology, and pathogenesis of human herpesvirus 8/Kaposi's sarcoma-associated herpesvirus and murine gammaherpesvirus 68 (MHV68). Dr Krug's laboratory examines the role of virus and host determinants in gammaherpesvirus pathogenesis using the MHV68 mouse pathogen model system. Her three major areas of investigation are: virus-host interactions that influence latency and viral gene expression in B cells; the role of tegument proteins in signaling and replication processes; and innate and adaptive immune mechanisms of host control and the virus counter-defense. Dr Krug was a member of the organizing committee for the recent National Institutes of Health Workshop 'Roseoloviruses: Clinical Impact, Interventions, and Research Needs.'

Four human herpesviruses were discovered in a eight-year period between 1986 and 1994. This exciting era of virus discovery was driven in part by the search for HIV and HIV-related diseases coupled with the development of new molecular tools such as PCR, automated Sanger sequencing, and subtractive hybridization. Three of these viruses, human herpesvirus 6A (HHV-6A) and human herpesvirus 6B (HHV-6B), and human herpesvirus 7 (HHV-7), were initially cultured from peripheral blood mononuclear cells. All three were found to be T lymphotropic viruses that were most closely related to human cytomegalovirus (HCMV), placing them in the betaherpesvirus family. Given their tight biologic and genetic relationships and clear etiologic link to roseola infantum, these viruses are now designated *Roseoloviruses*. Human herpesvirus 8 (also known as Kaposi's sarcoma associated herpesvirus) was identified as a new member of the gammaherpesviruses, in the rhadinovirus genus. Given its clear link to HIV-related malignancies, HHV-8 research exploded.

So began the struggle of the roseoloviruses for recognition and funding in the competitive world of biomedical research. The rate of discovery of their pathogenic potential has lagged compared to HHV-8 but great progress has been made nonetheless. This special section on the *Roseoloviruses* is intended to update the scientific community on the clinical impact, molecular virology, pathogenesis, and technological advancements in the field. The collection of reviews is a tangible product of a recent National Institutes of Health Workshop that brought roseolovirus experts together to discuss the clinical and basic science priorities of the field, summarized in the Perspective piece by Caserta *et al.* Each of these reviews highlights recent findings that address important aspects of 'roseolobiology' and each provides direction for further pursuits to fill-in specific gaps in knowledge.

Roseolovirus cytopathic effect is striking. Anyone who has witnessed the ballooning, refractile cells upon infection will wonder at the power of these viruses to cause such fundamental change in target cells. Krug and Pellett present an overview of unique features of the roseoloviruses and explore the genetic content of these viruses, pointing out the genes common to betaherpesviruses and those unique to the roseoloviruses. There are dozens of viral genes with unknown functions that will certainly provide important insight into the molecular basis of infection and disease. These gene products, in addition to newly discovered miRNAs, are untapped resources to understand how these viruses hijack reservoirs in the host such as T cells and astrocytes. A forward-thinking review of 'omics' technologies by

Moorman and Murphy provides a tantalizing look at how systems-based approaches might be applied to rapidly bring the molecular biology of roseoloviruses in line with other human herpesviruses. They advocate for genomics analysis of clinical isolates to establish reference strains and identify disease-associated variants, genome-wide gene expression studies to validate and classify the kinetics of transcripts, and functional screens of tagged-ORF expression libraries and BAC-based recombinant ORF mutant libraries coupled with proteomics to quickly ascribe gene function and viral protein interactions.

The review by Frenkel *et al.* is a telling story of how the roseoloviruses push the cell cycle into the G2/M phase and remarkably harness the E2F transcription factor to regulate the expression of the HHV-6A U27 and U79 genes. The unfolding mechanisms of virus subversion of both innate and adaptive immune responses is told by Amy Hudson. The roseolovirus repertoire includes gene products that target cytokine signaling, T cell activation, and downregulate MHC class I antigen presentation. Defining the role of viral immune modulators and uncharacterized genes will require experimentation in the whole animal to be realized. Horvat *et al.* summarize how the CD46-trangenic and humanized mouse models and non-human primate models recapitulate different aspects of roseolovirus disease in humans.

One striking feature of HHV-6A and HHV-6B is their ability to integrate into the telomeres of the human chromosome, in some cases resulting in heritable transmission of the viruses. Approximately 1% of the population harbors germline integrated HHV-6A or HHV-6B; chromosomal integration is a steadfast aspect of HHV-6A and HHV-6B biology. The review by Kaufer and Flamand describes recent advances in cell culture systems that allow researchers to examine how HHV-6A or HHV-6B integrate and excise themselves from host chromosomes. A pressing issue for the integration of HHV-6A and HHV-6B is determining if this is a requisite part of the virus lifecycle, potentially representing a novel mechanism for latency. Clearly, the clinical consequences of an integrated herpesvirus, whether in a few somatic cells or integrated into every cell of a human, requires further investigation.

Roseola infantum (*Exanthema subitum*) is a hallmark childhood illness comprised of a high fever lasting 1–5 days in duration that may be followed by a maculopapular rash. Tesini *et al.* summarize a series of clinical studies indicating that serious complications, such as febrile seizures and febrile seizure epilepticus, can arise from primary infection with HHV-6B and HHV-7. Human cytomegalovirus has long been associated with transplant complications, in part due to reactivation upon immunosuppression. As described by Hill and Zerr, allogeneic hematopoietic stem cell transplant patients, and in particular cord blood stem cell recipients, are at higher risk of HHV-6B reactivation associated with limbic encephalitis and neurocognitive disorder. A balanced review of the current literature regarding the association of the neurotropic roseoloviruses with multiple sclerosis (MS) is presented by Leibovitch and Jacobsen. Evidence for both direct roles of the virus and virus-driven immune responses in MS pathology are discussed.

Diagnosis of primary roseolovirus infection and CNS-related complications arising from both primary infections and reactivation in immunosuppressed transplant patients would benefit from rapid diagnostics and less toxic antiviral drugs. Hill *et al.* introduce the use of digital PCR to distinguish single integrated viral genomes per cell in patients with chromosomal integration of HHV-6A or HHV-6B (ciHHV-6) from a high copy number of virus in a blood sample due to viral reactivation. This review also highlights the importance of using other molecular tools such as quantitative reverse-transcript PCR of mRNA to distinguish latent from active, lytic infections.

Clinicians need safe and effective therapies to control roseolovirus infection and limit viral pathogenesis. The efficacy of current nucleoside analogs and of drugs in the developmental pipeline is reviewed by Prichard and Whitley. The authors point out that there is little fiscal incentive for the pharmaceutical industry to dedicate research and development to the roseoloviruses without clear disease etiology. However, Koch's postulates are difficult to fulfill for ubiquitious viruses. In a frustrating case of 'Catch-22', clinical trials with roseolovirus-specific drug therapies are key to demonstrating that virus infection leads to the resolution of a suspected roseolovirus-associated disease. Immune therapy shows real promise in the treatment of reactivation-associated disease in transplant recipients. Becerra *et al.* define the predominant HHV-6A and HHV-6B epitopes that CD4+ T cells and CD8+ T cells recognize and explain how these T cells can be expanded in culture for autologous transfer and protection.

These last several years have produced a collection of new data, technologies, and ideas that generates important new questions about roseolovirus biology. Can we treat reactivation and nervous system disease with novel antivirals and immune therapy? Does an integrated virus place a patient at risk for disease? Is integration a part of the virus lifecycle? What are the functions of uncharacterized gene products during infection, and how do they impact pathogenesis? Can we confirm or discount roseolovirus causality or contributions to rare or complex diseases? We direct the reader to the focused reviews on the molecular and clinical aspects of HHV-6A, HHV-6B, and HHV-7 in this special section on the *Roseoloviruses*. It is time to stop and smell the roses.

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Current Opinion in Virology

Roseolovirus molecular biology: recent advances

Laurie T Krug[1] and Philip E Pellett[2]

Human herpesviruses 6A, 6B, and 7 (HHV-6A, HHV-6B, and HHV-7) are classified within the roseolovirus genus of the betaherpesvirus subfamily. Most humans likely harbor at least two of these large DNA viruses, and 1% of humans harbor germline chromosomally integrated (ci) HHV-6A or HHV-6B genomes. Differences at the genetic level manifest as distinct biologic properties during infection and disease. We provide a brief synopsis of roseolovirus replication and highlight the unique properties of their lifecycle and what is known about the viral gene products that mediate these functions. In the nearly 30 years since their discovery, we have only begun to unlock the molecular strategies these highly evolved pathogens employ to establish and maintain chronic infections in humans.

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Edited by **Laurie Krug**

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The aims of this review are to provide an overview of roseolovirus molecular biology and highlight recent advances in our understanding of the molecular basis of the virus lifecycle, which in turn inform our understanding of pathogenesis, and illuminate paths to diagnosis, treatment, and prevention.

Roseoloviruses: what are they?

Human herpesviruses 6A, 6B, and 7 (HHV-6A, HHV-6B, and HHV-7) are the only formally recognized members of genus *Roseolovirinae* within order *Herpesvirales*, family *Herpesviridae*, and subfamily *Betaherpesvirales* (Figure 1) (historical references are available in [1,2]). HHV-6A and HHV-6B were formerly described as variants, but are now formally classified as distinct virus species by the International Committee on Virus Taxonomy [3]. Roseoloviruses share numerous genetic and biologic properties with human cytomegalovirus (also a betaherpesvirus), yet have distinct genes and disease associations (Tables 1 and 2). The human roseoloviruses are contemporary representatives of an ancient lineage of herpesviruses that cospeciated with their hosts. Antibodies against HHV-6 have been detected in several species of Old and New World monkeys, suggesting the presence of viruses related to HHV-6 in these animals [4]. Consistent with this, relatives of HHV-6 and HHV-7 have been detected by PCR in chimpanzees, other great apes, and pig-tailed macaques [5–7].

Roseoloviruses and human health

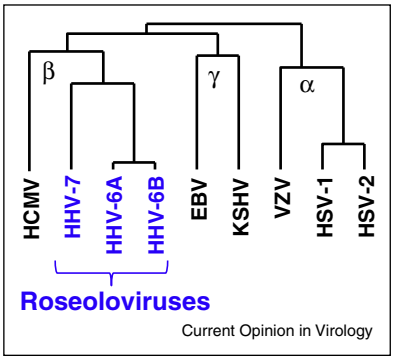
HHV-6B is the most common cause of roseola infantum (exanthem subitum) and related febrile rash illnesses that often accompany primary infection in early childhood [8]; this can also be caused by HHV-7. HHV-6B and HHV-7 have also been associated with febrile seizures in young children. Immune suppressed hemopoietic stem cell transplant recipients can experience limbic encephalitis and other mental disorders during HHV-6B reactivations [9]. HHV-6A has been associated with Hashimoto's thyroiditis [10] and neurological disorders, including multiple sclerosis, but proof of causality is incomplete [11].

A striking feature of roseoloviruses is the presence of mammalian telomeric sequences at the ends of the virus genome [12,13••,14]. Approximately 1% of the human population world-wide harbors inherited chromosomally integrated (ci) HHV-6A and HHV-6B. Germline integration may be a byproduct of the use of integration as a hypothesized mechanism for establishing latency in somatic cells, with virus infection of spermatocytes leading to occasional germline transmission. The health effects of ciHHV-6 have not been elucidated.

Roseolovirus genomes and genes

Roseoloviruses genomes consist of a long unique region (U) bracketed by a pair of direct repeats (DR) (Figure 2). Roseolovirus genomes have heterogeneous and perfect arrays of mammalian telomeric repeats at the left and right ends of the DR elements, respectively, and consequently at the left and right genomic termini. At least for HHV-6B, genomes of wild viruses can be several kb longer than those of laboratory-adapted strains, due to repetitive sequences in the DR that are lost upon passage in cultured cells. Roseolovirus genomes are approximately 65–90 kb shorter than the 235 kb HCMV genome. The origins of lytic genome replication (oriLyt) are located between U41 and U42, and are structurally similar to oriLyts of alphaherpesviruses.

Figure 1



Dendrogram showing relationships among the human herpesviruses, based on sequences of the conserved protein, gB.

HHV-6A and HHV-6B are ~90% identical across their genomes, with ~95% identity across the herpesvirus core genes. Regions in the vicinity of the genomic termini are less conserved, with as little as 50% identity in the region that encodes the major immediate early transactivators [15]. While its overall organization and gene content are similar to those of HHV-6A and HHV-6B, the HHV-7 genome is shorter and more compactly arranged across its length, with many genes being 5–10% shorter than their HHV-6 counterparts. In intrastrain comparisons, roseolovirus genomes are typically ~99.9% identical, except for pockets of elevated heterogeneity.

The core herpesvirus genes (43 genes conserved among members of the *Herpesviridae*) are clustered across the central portion of the genomes in an arrangement colinear with the core genes in HCMV and other betaherpesviruses.

Table 1

Genetic and biological properties of human roseoloviruses and HCMV

	HHV-6A	HHV-6B	HHV-7	HCMV
Commonly used strains	U1102, GS	Z29, HST	JI, RK, SB, UCL-1	AD169, Towne, Merlin, TB40E
Length of wild genomes	?	~170 kb	?	236 kb
Length of passaged genomes	159 kb	159-162 kb	145 kb	~230 kb
genes encoding unique proteins	~102	~97	~86	~165
miRNAs	4 predicted	4	unknown	16
Replication	slow, extended			
	ballooning, refractile cytopathic effect			cytomegaly, nuclear and cytoplasmic inclusions
	origin-binding protein for initiation of DNA replication			
Cell surface receptor	CD46	CD134	CD4	EGFR, Integrins
Cell culture tropism	umbilical cord blood lymphocytes peripheral blood mononuclear cells			monocyte-macrophages
	T cell lines: SupT-1, HSB2, J JAHN	T cell lines: Molt-3, Mt-4, SupT-1	T cell lines: SupT-1	CD34+ hematopoietic cells
	productive replication in astrocytes	low-level persistence in astrocytes		endothelial and epithelial cells, fibroblasts
Unique features	integration into host telomeres			
Major disease associations	Hashimoto's thyroiditis	exanthem subitum		congenital birth defects
		febrile seizures/ status epilepticus		transplant complications
		transplant complications		retinitis
	post-transplant reactivation-associated encephalitis			hepatitis

Table 2

Genes unique to roseoloviruses

Function[a]	Roseolovirus ORF	%S with HHV-6A[b]	%I with HHV-6A[c]	%S with HHV-7	%I with HHV-7
Roseolovirus specific genes					
	U13	93.4	92.5	44.9	35.7
	U15EX1	91.4	86.7	76.4	67.9
	U15EX2	100	95.8	83.3	75
	U15EX3	96.7	91.7	82	75.4
Glycoprotein	U20	95.6	95.6	31.8	22.2
Downregulation of MHC class I	U21	91	89.8	42.8	31.6
Glycoprotein	U23	94.6	94.1	26.9	20.9
	U24	88.3	82.7	46.2	31.2
	U24A	94.7	91.2	40.3	28.1
	U26	93.8	92.9	60.5	47.4
OX-2 homology, glycoprotein	U85	93.1	91.7	46.9	36.8
IE-A (IE1), transactivator	U90EX1	73.7	68.4	42.8	35.7
	U90EX2	70.3	67.2	67.1	57.1
	U90EX3	76.7	71.5	32.9	25.2
IE-A	U91EX1	67.8	57.1	33.3	25
	U91EX2	69.2	67.9	45.6	40
Spliced envelope glycoprotein; HHV-6 gp82-gp105, HHV-7 gp65	U100EX1	78.1	73.4	27.2	19.7
	U100EX2	84.9	81.7	53.8	38.7
	U100EX3	82.9	79.3	40.9	32.7
	U100EX4	96	88	44	40
	U100EX5	88.6	80	34.3	28.6
	U100EX6	91.9	91.9	48.6	37.8
	U100EX7	88.7	83	35.3	27.4
	U100EX8	100	100		
	U100EX9	92.8	90.5	35.7	23.8
	U100EX10	83.9	76.5	24	13.3
HHV-6 specific genes					
	DR3	87	86.4		
	U6	97.1	97.1		
	U9	94.2	94.2		
Glycoprotein	U22	91.2	89.6		
Intercrine cytokine	U83	87.6	85.6		
Parvovirus *rep* homolog	U94	98.4	97.6		
HHV-6A gene					
	U78				
HHV-6B genes					
	B3, B4, B5, B6, B7, B8				

[a] Implied functions of homologous genes or experimental validation.
[b] Percentage of amino acid similarity between homologs in comparison to HHV-6B strain Z29.
[c] Percentage of amino acid identity between homologs in comparison to HHV-6B strain Z29.

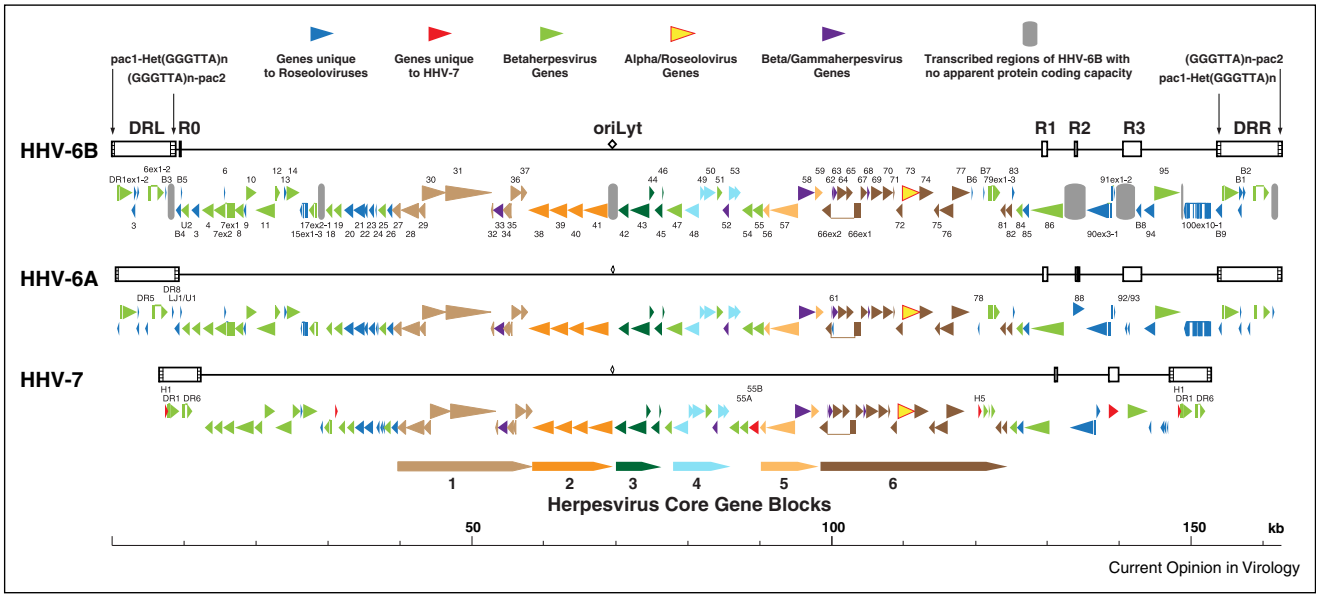
In contrast to HCMV and most other betaherpesviruses, the roseoloviruses, along with elephant endotheliotropic herpesviruses, encode homologs (roseolovirus gene U73) of the origin of DNA replication binding protein (OBP) encoded by all alphaherpesviruses. Most of the genes shared only among betaherpesviruses or unique to one or more roseoloviruses lie in or near the DR, or between conserved gene blocks (Table 2 and Figure 2).

HHV-6B expresses several small RNAs of unknown function, including some that map to oriLyt and microRNAs that map to the DR3/B1 and B2 immediate early gene locus in DR. These miRNAs are conserved in HHV-6A, and one is an ortholog of human miRNA miR-582-5p [16••].

Major functions of many roseolovirus genes are known only by inference from known functions of their homologs in HCMV or other herpesviruses. Most virion proteins are likely to have significant biological roles that go beyond structural, such as tegument proteins that modify host cell activities before *de novo* viral gene expression begins. Only a handful of genes unique to roseoloviruses have been studied functionally. These include transactivators encoded by DR6 and U3, the U94 parvovirus rep gene homolog, immunoevasins encoded by U21, a nonessential Golgi-localizing nonstructural glycoprotein encoded by U23 [17], and the gQ1 and gQ2 glycoproteins.

Major research priorities include assessment of genome sequences and genetic variation of wild viruses, and

Figure 2



Genomic and genetic architectures of the human roseoloviruses.
On the basis of information from [15,63–67].

identification of the functions of genes unique to roseoloviruses. A bacterial artificial chromosome (BAC) system has enabled targeted genetic analysis for HHV-6A [18]; analogous systems are needed for HHV-6B and HHV-7.

Productive replication

Roseolovirus tropism: beyond T cells

The human roseoloviruses were discovered on the basis of their lytic replication activity in cultured PBMCs. Some strains have adapted to growth in specific T cell lines and are commonly used for laboratory studies. Other cell types such as monocytes, dendritic cells, astrocytes, and glial cells are permissive for infection. HHV6A and HHV-6B can bind to the sperm acrosome, providing a possible route to germline integration [19]. The ability of HHV-6A and HHV-6B to infect olfactory-ensheathing glial cells that are present in the nasal cavity may provide a route to the central nervous system [20].

Mechanisms of attachment and entry are important determinants of cell tropism and latency reservoirs in the host. Each roseolovirus has a distinct entry receptor: CD46 for HHV-6A [21], CD134 for HHV-6B [22•], and CD4 for HHV-7 [23]. Receptors are targets for neutralization [24] and can be used to create receptor-transgenic animal models that support infection [25•]. The essential components for membrane fusion by HHV-6A and HHV-6B are gB and the gH/gL/gQ1/gQ2 complex [26,27,28•]. gQ2 and gM are essential for virus production of HHV-6A since virus stocks could not be generated from BACs with disruptions in these ORFs [28•,29]. The degree of functional homology between roseolovirus genes can be examined in transcomplementation assays and by gene substitutions in the HHV-6A BAC. For instance, the HHV-6B gH gene can functionally replace HHV-6A gH for replication [30].

De novo gene expression and productive replication

Roseolovirus lytic gene expression follows the general herpesvirus paradigm: immediate early genes are transcribed in the absence of new protein synthesis, expression of early genes is dependent on prior synthesis or immediate early proteins, and late genes are expressed at high levels upon viral DNA replication. Approximately 10 genes have spliced transcripts (some have multiple spliced isoforms), and some transcripts are kinetically regulated. Roseolovirus major IE genes are spliced and have promoters that can be highly active in T cells.

Roseoloviruses diverge from most betaherpesviruses in their mechanism of initiating viral DNA replication. Their homologs of the alphaherpesvirus origin binding protein bind to, and presumably facilitate unwinding of the origin of lytic replication to initiate viral DNA synthesis [31]. The OBPs of HHV-6B and HHV-7 have slight differences in preferential binding sites that may explain a lack of complete reciprocity between HHV-6B and HHV-7 in transient oriLyt replication assays.

Information about HHV-6 virion assembly and egress is sparse. An interesting feature of HHV-6A virion

envelopes is the presence of ganglioside GM1, a component of lipid rafts [32]. Along with other evidence, this suggests that virions may assemble via lipid rafts. Envelopment and egress are via a cellular CD63-associated exosomal pathway [33].

Latency and reactivation

Gene expression during latency

Roseolovirus latency is poorly defined in molecular terms. CD34-positive hematopoietic cells are a site of HHV-6 latency, and circulating lymphocytes positive for HHV-7 DNA but not for lytic gene transcripts have been detected. Latency associated transcripts have been identified in two loci: antisense to the major IE locus, with splicing patterns reminiscent of an HCMV latency transcript [34], and from the U94 gene [35]. No laboratory has reported detection of both of these transcripts.

Integration

One of the most unique and biologically intriguing aspects of HHV-6A and HHV-6B is their integration into the germline of some humans (~1%), which can result in inherited transmission among families [14]. All three human roseoloviruses contain mammalian telomeric sequences at their genomic termini, and telomeres are the site of integration of HHV-6A and HHV-6B in patients with chromosomally integrated HHV-6 [36,37••]. Telomeric integration occurs in infected cultured Jjhan and HEK-293 cells, establishing a system for mapping and characterizing the mechanistic processes of integration. The efficiency of integration in cultured cells has led to the hypothesis that chromosomal integration is a normal part of HHV-6 latency.

The U94 gene of HHV-6A and HHV-6B is a homolog of the parvovirus Rep gene, an integrase with single-stranded and double-stranded DNA binding properties. Cytomegaloviruses of rats [38] and bats [39] encode U94 homologs, indicating that the gene may have been acquired prior to the divergence of roseoloviruses and cytomegaloviruses. HHV-6 U94 binds ssDNA [40] and its ectopic expression inhibits betaherpesvirus replication [41] and impairs lymphatic endothelial cell angiogenesis [42]. Given its homology with the parvovirus integrase, U94 is hypothesized to promote integration and excision of HHV-6A and HHV-6B, either by host-mediated base excision repair or by exonuclease strand invasion [14]. The transcriptome of ciHHV-6 cells has not been reported, but spliced U90 transcripts have been detected in B cells harboring integrated HHV-6 [43•]. Genome-wide analyses of viral and cellular gene expression are needed in individuals with ciHHV-6 and in ciHHV-6 cell culture systems.

Reactivation

Uncontrolled or aberrant primary infection and HHV-6 reactivation are associated with neurological syndromes and transplant failure. Very little is known about the molecular basis of reactivation. Mitogen stimulation of PBMCs leads to reactivation and enables infection of T cell lines. Lytic replication can also be stimulated by apoptosis [44]. If integration is a mechanism of latency, a functional virus genome must be excised from telomeres in order to reactivate full lytic infection. HEK293 cells with integrated HHV-6A can produce viral genome concatamers upon treatment with the histone deacetylase inhibitor trichostatin A [36]. Huang *et al.* [43•] noted that the telomeres attached to integrated HHV-6 genomes are frequently shortened and associated with detection of circular viral genomes. Such short, unstable telomeres are thought to facilitate excision of viral genomes via telomere-loops within the viral genome [43•]. Interestingly, *Chlamydia trachomatis* drives reactivation of ciHHV-6 and transient shortening of telomere ends [45]; the signaling pathways and mechanism of excision remain to be defined.

Virus–host interactions

All herpesviruses manipulate host cell processes to promote replication. Roseoloviruses push the cell cycle into G2/M, presumably to ramp up cellular processes that promote DNA replication [46]. Virally induced degradation of Rb and activation of E2F1 further benefits HHV-6A and HHV-6B by enhancing the expression of some lytic genes [47]. Many roseolovirus gene products inhibit both innate immune responses (U20, IE1) and adaptive immune responses (U21), and interfere with cell death (U19, U20, DR6) and T cell signaling (U21, U54) [48]. Functions should not be assumed to be conserved among all roseolovirus homologs. Virus-specific differences in gene function such as U54 modulation of IL-2 signaling, the chemotactic properties of the roseolovirus U83 chemokines, and IE1 inhibition of interferon stimulated genes have been noted [49–51]. BAC-based recombinant viruses will facilitate examination of gene function in the context of infection.

Roseoloviruses impact cytokine profiles of cultured cells [52,53]. Cytokine dysregulation also occurs in patients undergoing acute illness associated with primary infection [54–56] and reactivation [57], and in animal models of HHV-6 infection [25•,58]. The viral gene products that induce these changes in host signaling are not known. Inactivated virions induce an interferon-lambda 1 (IL-29) response in dendritic cells that might skew T cell responses to infection [59]. The host immune response may play a large role in the immune pathology of reactivation-associated diseases and facilitate roseolovirus transit across the blood brain barrier [60]. In addition, HHV-6B reactivation might be triggered in response to pro-inflammatory cytokines such as TNF-alpha and immunosuppression with corticosteroids. Such a mechanism might contribute to the frequent detection of HHV-6B reactivation in patients diagnosed with Drug

Reaction with Eosinophilia and Systemic Symptom (DRESS), a potential fatal syndrome initiated by adverse drug reactions [61,62].

Understanding the functional changes described above will be enhanced by deep analysis of the effects of roseolovirus infection on host cell transcription, translation, and export of gene products.

Research priorities

Understanding of the molecular virology of roseoloviruses lags behind that for all other human herpesviruses. Understanding the genetic content of roseoloviruses has not been extended far beyond basic sequence analysis of laboratory-adapted strains. Modern methods of DNA sequencing need to be applied to understanding the sequence composition of wild, uncultured roseolovirus genomes, as well as interhost and intrahost sequence variation at the genome level in immune competent and immune compromised individuals. Among other things, such genetic analyses are necessary to ensure that animal studies and other experiments are done with viruses that appropriately represent wild viruses. Functional analysis of the genes unique to roseoloviruses and betaherpesviruses will provide information as to how these viruses have adapted to their specific and specialized niches. Genetic approaches using BAC-based recombination strategies are critical to identify the viral factors and *cis*-determinants of replication, integration, and reactivation. Even in the absence of well-established genetically tractable systems for HHV-6B and HHV-7, transcript and proteomic profiles can rapidly confirm putative genes and identify novel ORFs, novel transcript forms, and noncoding RNAs. Vaccine development typically involves attenuation, but intelligently designed attenuation will not be possible for the roseoloviruses without fundamental knowledge of replication and host interaction determinants.

Summary

Roseolovirues have unique cellular tropisms and biological properties, and encode ORFs distinct from the other human betaherpesvirus, HCMV. Each HHV-7 gene has a homolog in HHV-6A and HHV-6B. However, HHV-6A and HHV-6B have several genes not found in HHV-7, including a homolog of the parvovirus rep protein, U94. Roseolovirus gene products mediate cell entry and viral replication, modulate the host cell's growth, survival, signaling, and immune responses, and regulate latency. RNA analyses and proteomics coupled with new genetic tools and advances in systems biology are needed to advance the identification and function of known, as well as uncharacterized and novel ORFs, and transcripts such as miRNAs. Advancements in understanding roseolovirus gene function will reveal novel virus–host interactions and better define the mechanism of integration and excision of the virus genome into and from host chromosomes, a potential form of latency that would be unique among the human herpesviruses. Investments in understanding the fundamental molecular processes of roseolovirus infections will inform our understanding of the dynamic process of persistence and disease in humans and identify targets for therapeutic intervention.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Braun DK, Dominguez G, Pellett PE: **Human herpesvirus 6**. *Clin Microbiol Rev* 1997:521-567.

2. Yamanishi K, Mori Y, Pellett PE: **Human herpesviruses 6 and 7**. In *Fields Virology*, vol 2. Edited by Knipe DM, Howley PM, Cohen JI, Griffin DE, Lamb RA, Martin MA, Racaniello VR, Roizman B. Lippincott Williams & Wilkins; 2013:2058-2079 (Chapter 64).

3. Ablashi D, Agut H, Alvarez-Lafuente R, Clark DA, Dewhurst S, DiLuca D, Flamand L, Frenkel N, Gallo R, Gompels UA *et al.*: **Classification of HHV-6A and HHV-6B as distinct viruses**. *Arch Virol* 2014, **159**:863-870 http://dx.doi.org/10.1007/s00705-013-1902-5.

4. Higashi K, Asada H, Kurata T, Ishikawa K, Hayami M, Spriatna Y, Sutarman, Yamanishi K: **Presence of antibody to human herpesvirus 6 in monkeys**. *J Gen Virol* 1989, **70(Pt 12)**:3171-3176.

5. Lacoste V, Verschoor EJ, Nerrienet E, Gessain A: **A novel homologue of Human herpesvirus 6 in chimpanzees**. *J Gen Virol* 2005, **86**:2135-2140 http://dx.doi.org/10.1099/vir.0.81034-0.

6. Lavergne A, Donato D, Gessain A, Niphuis H, Nerrienet E, Verschoor EJ, Lacoste V: **African great apes are naturally infected with roseoloviruses closely related to Human herpesvirus 7**. *J Virol* 2014 http://dx.doi.org/10.1128/JVI.01490-14.

7. Staheli JP, Dyen MR, Lewis P, Barcy S: **Discovery and biological characterization of two novel pig-tailed macaque homologs of HHV-6 and HHV-7**. *Virology* 2014, **471-473**:126-140 http://dx.doi.org/10.1016/j.virol.2014.10.008.

8. Tesini B, Epstein LG, Caserta MT: **Clinical impact of primary infection with roseoloviruses**. *Curr Opin Virol* 2014, **9C**.

9. Hill J, Zerr DM: **Roseoloviruses in transplant recipients: clinical consequences and prospects for treatment and prevention trials**. *Curr Opin Virol* 2014, **9C**.

10. Caselli E, Zatelli MC, Rizzo R, Benedetti S, Martorelli D, Trasforini G, Cassai E, degli Uberti EC, Di Luca D, Dolcetti R: **Virologic and immunologic evidence supporting an association between HHV-6 and Hashimoto's thyroiditis**. *PLoS Pathog* 2012, **8**:e1002951 http://dx.doi.org/10.1371/journal.ppat.1002951.

11. Leibovitch E, Jacobson S: **An update on the evidence linking HHV-6 with multiple sclerosis**. *Curr Opin Virol* 2014, **9C**.

12. Luppi M, Marasca R, Barozzi P, Ferrari S, Ceccherini-Nelli L, Batoni G, Merelli E, Torelli G: **Three cases of human herpesvirus-6 latent infection: integration of viral genome in peripheral blood mononuclear cell DNA**. *J Med Virol* 1993, **40**:44-52.

13. •• Pellett PE, Ablashi DV, Ambros PF, Agut H, Caserta MT, Descamps V, Flamand L, Gautheret-Dejean A, Hall CB, Kamble RT *et al.*: **Chromosomally integrated human herpesvirus**

6: questions and answers. *Rev Med Virol* 2012, **22**:144-155 http://dx.doi.org/10.1002/rmv.715.
An international collaboration of many experts in the field that addresses the consequences of HHV-6A and HHV-6B integration into the host chromosome.

14. Kaufer BB, Flamand L: **Chromosomally integrated HHV-6: impacts on virus, cell and organismal biology**. *Curr Opin Virol* 2014, **9C**.

15. Dominguez G, Dambaugh TR, Stamey FR, Dewhurst S, Inoue N, Pellett PE: **Human herpesvirus 6B genome sequence: coding content and comparison with human herpesvirus 6A**. *J Virol* 1999, **73**:8040-8052.

16. •• Tuddenham L, Jung JS, Chane-Woon-Ming B, Dölken L, Pfeffer S: **Small RNA deep sequencing identifies microRNAs and other small noncoding RNAs from human herpesvirus 6B**. *J Virol* 2012, **86**:1638-1649 http://dx.doi.org/10.1128/JVI.05911-11.
Identification of HHV-6B miRNAs and other noncoding RNAs expressed during lytic infection.

17. Hayashi M, Yoshida K, Tang H, Sadaoka T, Kawabata A, Jasirwan C, Mori Y: **Characterization of the human herpesvirus 6A U23 gene**. *Virology* 2014, **450/451**:98-105 http://dx.doi.org/10.1016/j.virol.2013.12.004.

18. Tang H, Kawabata A, Yoshida M, Oyaizu H, Maeki T, Yamanishi K, Mori Y: **Human herpesvirus 6 encoded glycoprotein Q1 gene is essential for virus growth**. *Virology* 2010, **407**:360-367 http://dx.doi.org/10.1016/j.virol.2010.08.018.

19. Kaspersen MD, Larsen PB, Kofod-Olsen E, Fedder J, Bonde J, Höllsberg P: **Human herpesvirus-6A/B binds to spermatozoa acrosome and is the most prevalent herpesvirus in semen from sperm donors**. *PLoS ONE* 2012, **7**:e48810 http://dx.doi.org/10.1371/journal.pone.0048810.

20. Harberts E, Yao K, Wohler JE, Maric D, Ohayon J, Henkin R, Jacobson S: **Human herpesvirus-6 entry into the central nervous system through the olfactory pathway**. *Proc Natl Acad Sci U S A* 2011, **108**:13734-13739 http://dx.doi.org/10.1073/pnas.1105143108.

21. Santoro F, Kennedy PE, Locatelli G, Malnati MS, Berger EA, Lusso P: **CD46 is a cellular receptor for human herpesvirus 6**. *Cell* 1999, **99**:817-827.

22. • Tang H, Serada S, Kawabata A, Ota M, Hayashi E, Naka T, Yamanishi K, Mori Y: **CD134 is a cellular receptor specific for human herpesvirus-6B entry**. *Proc Natl Acad Sci U S A* 2013, **110**:9096-9099 http://dx.doi.org/10.1073/pnas.1305187110.
Discovery of the HHV-6B cellular entry receptor.

23. Lusso P, Secchiero P, Crowley RW, Garzino-Demo A, Berneman ZN, Gallo RC: **CD4 is a critical component of the receptor for human herpesvirus 7: interference with human immunodeficiency virus**. *Proc Natl Acad Sci U S A* 1994, **91**:3872-3876.

24. Kawabata A, Oyaizu H, Maeki T, Tang H, Yamanishi K, Mori Y: **Analysis of a neutralizing antibody for human herpesvirus 6B reveals a role for glycoprotein Q1 in viral entry**. *J Virol* 2011, **85**:12962-12971 http://dx.doi.org/10.1128/JVI.05622-11.

25. • Reynaud JM, Jégou J-F, Welsch JC, Horvat B: **Human herpesvirus 6A infection in CD46 transgenic mice: viral persistence in the brain and increased production of proinflammatory chemokines via Toll-like receptor 9**. *J Virol* 2014, **88**:5421-5436 http://dx.doi.org/10.1128/JVI.03763-13.
Development of an animal model based on mice that express human CD46, the HHV-6A receptor, with evidence of HHV-6A in the brain.

26. Maeki T, Hayashi M, Kawabata A, Tang H, Yamanishi K, Mori Y: **Identification of the human herpesvirus 6A gQ1 domain essential for its functional conformation**. *J Virol* 2013, **87**:7054-7063 http://dx.doi.org/10.1128/JVI.00611-13.

27. Maeki T, Mori Y: **Features of human herpesvirus-6A and -6B entry**. *Adv Virol* 2012:384069 http://dx.doi.org/10.1155/2012/384069.2012.

28. • Tang H, Hayashi M, Maeki T, Yamanishi K, Mori Y: **Human herpesvirus 6 glycoprotein complex formation is required for folding and trafficking of the gH/gL/gQ1/gQ2 complex and its cellular receptor binding**. *J Virol* 2011, **85**:11121-11130 http://dx.doi.org/10.1128/JVI.05251-11.
Development and application of an HHV-6A BAC suitable for genetic analysis of viral gene functions.

29. Kawabata A, Jasirwan C, Yamanishi K, Mori Y: **Human herpesvirus 6 glycoprotein M is essential for virus growth and requires glycoprotein N for its maturation**. *Virology* 2012, **429**:21-28 http://dx.doi.org/10.1016/j.virol.2012.03.027.

30. Oyaizu H, Tang H, Ota M, Takenaka N, Ozono K, Yamanishi K, Mori Y: **Complementation of the function of glycoprotein H of human herpesvirus 6 variant A by glycoprotein H of variant B in the virus life cycle**. *J Virol* 2012, **86**:8492-8498 http://dx.doi.org/10.1128/JVI.00504-12.

31. Krug LT, Inoue N, Pellett PE: **Sequence requirements for interaction of human herpesvirus 7 origin binding protein with the origin of lytic replication**. *J Virol* 2001, **75**:3925-3936 http://dx.doi.org/10.1128/JVI.75.8.3925-3936.2001.

32. Kawabata A, Tang H, Huang H, Yamanishi K, Mori Y: **Human herpesvirus 6 envelope components enriched in lipid rafts: evidence for virion-associated lipid rafts**. *Virol J* 2009, **6**:127 http://dx.doi.org/10.1186/1743-422X-6-127.

33. Mori Y, Koike M, Moriishi E, Kawabata A, Tang H, Oyaizu H, Uchiyama Y, Yamanishi K: **Human herpesvirus-6 induces MVB formation, and virus egress occurs by an exosomal release pathway**. *Traffic* 2008, **9**:1728-1742 http://dx.doi.org/10.1111/j.1600-0854.2008.00796.x.

34. Kondo K, Shimada K, Sashihara J, Tanaka-Taya K, Yamanishi K: **Identification of human herpesvirus 6 latency-associated transcripts**. *J Virol* 2002, **76**:4145-4151.

35. Rotola A, Ravaioli T, Gonelli A, Dewhurst S, Cassai E, Di Luca D: **U94 of human herpesvirus 6 is expressed in latently infected peripheral blood mononuclear cells and blocks viral gene expression in transformed lymphocytes in culture**. *Proc Natl Acad Sci U S A* 1998, **95**:13911-13916.

36. Arbuckle JH, Pantry SN, Medveczky MM, Prichett J, Loomis KS, Ablashi D, Medveczky PG: **Mapping the telomere integrated genome of human herpesvirus 6A and 6B**. *Virology* 2013, **442**:3-11 http://dx.doi.org/10.1016/j.virol.2013.03.030.

37. •• Arbuckle JH, Medveczky MM, Luka J, Hadley SH, Luegmayr A, Ablashi D, Lund TC, Tolar J, De Meirleir K, Montoya JG *et al.*: **The latent human herpesvirus-6A genome specifically integrates in telomeres of human chromosomes in vivo and in vitro**. *Proc Natl Acad Sci U S A* 2010, **107**:5563-5568 http://dx.doi.org/10.1073/pnas.0913586107.
This landmark paper described the telomeric targets of HHV-6A chromosomal integration in human specimens and in a cell culture model.

38. van Cleef KWR, Scaf WMA, Maes K, Kaptein SJF, Beuken E, Beisser PS, Stassen FRM, Grauls GELM, Bruggeman CA, Vink C: **The rat cytomegalovirus homologue of parvoviral rep genes, r127, encodes a nuclear protein with single- and double-stranded DNA-binding activity that is dispensable for virus replication**. *J Gen Virol* 2004, **85**:2001-2013 http://dx.doi.org/10.1099/vir.0.79864-0.

39. Zhang H, Todd S, Tachedjian M, Barr JA, Luo M, Yu M, Marsh GA, Crameri G, Wang L-F: **A novel bat herpesvirus encodes homologues of major histocompatibility complex classes I and II, C-type lectin, and a unique family of immune-related genes**. *J Virol* 2012, **86**:8014-8030 http://dx.doi.org/10.1128/JVI.00723-12.

40. Dhepakson P, Mori Y, Jiang YB, Huang HL, Akkapaiboon P, Okuno T, Yamanishi K: **Human herpesvirus-6 rep/U94 gene product has single-stranded DNA-binding activity**. *J Gen Virol* 2002, **83**:847-854.

41. Caselli E, Bracci A, Galvan M, Boni M, Rotola A, Bergamini C, Cermelli C, Dal Monte P, Gompels UA, Cassai E *et al.*: **Human herpesvirus 6 (HHV-6) U94/REP protein inhibits betaherpesvirus replication**. *Virology* 2006, **346**:402-414 http://dx.doi.org/10.1016/j.virol.2005.11.018.

42. Caruso A, Caselli E, Fiorentini S, Rotola A, Prandini A, Garrafa E, Saba E, Alessandri G, Cassai E, Di Luca D: **U94 of human herpesvirus 6 inhibits in vitro angiogenesis and**

lymphangiogenesis. *Proc Natl Acad Sci U S A* 2009, **106**:20446-20451 http://dx.doi.org/10.1073/pnas.0905535106.

43. • Huang Y, Hidalgo-Bravo A, Zhang E, Cotton VE, Mendez-Bermudez A, Wig G, Medina-Calzada Z, Neumann R, Jeffreys AJ, Winney B *et al.*: **Human telomeres that carry an integrated copy of human herpesvirus 6 are often short and unstable, facilitating release of the viral genome from the chromosome**. *Nucleic Acids Res* 2014, **42**:315-327 http://dx.doi.org/10.1093/nar/gkt840.
Discovery that somatic cell chromosomes with integrated HHV-6 have short telomeres, and evidence that telomeric loop formation is involved in excision of ciHHV-6 genomes during viral reactivation.

44. Prasad A, Remick J, Zeichner SL: **Activation of human herpesvirus replication by apoptosis**. *J Virol* 2013, **87**:10641-10650 http://dx.doi.org/10.1128/JVI.01178-13.

45. Prusty BK, Siegl C, Hauck P, Hain J, Korhonen SJ, Hiltunen-Back E, Puolakkainen M, Rudel T: ***Chlamydia trachomatis* infection induces replication of latent HHV-6**. *PLOS ONE* 2013, **8**:e61400 http://dx.doi.org/10.1371/journal.pone.0061400.

46. Shamir E, Zeigerman H, Frenkel N: **Roseoloviruses: viral host interactions and cell cycle arrest**. *Curr Opin Virol* 2014, **9C**.

47. Sharon E, Volchek L, Frenkel N: **Human herpesvirus 6 (HHV-6) alters E2F1/Rb pathways and utilizes the E2F1 transcription factor to express viral genes**. *Proc Natl Acad Sci U S A* 2014, **111**:451-456 http://dx.doi.org/10.1073/pnas.1308854110.

48. Hudson A: **Roseoloviruses: a treasure trove of immune modulators**. *Curr Opin Virol* 2014, **9C**.

49. Iampietro M, Morissette G, Gravel A, Flamand L: **Inhibition of interleukin-2 gene expression by human herpesvirus 6B U54 tegument protein**. *J Virol* 2014, **88**:12452-12463 http://dx.doi.org/10.1128/JVI.02030-14.

50. Clark DJ, Catusse J, Stacey A, Borrow P, Gompels UA: **Activation of CCR2+ human proinflammatory monocytes by human herpesvirus-6B chemokine N-terminal peptide**. *J Gen Virol* 2013, **94**:1624-1635 http://dx.doi.org/10.1099/vir.0.050153-0.

51. Jaworska J, Gravel A, Flamand L: **Divergent susceptibilities of human herpesvirus 6 variants to type I interferons**. *Proc Natl Acad Sci U S A* 2010, **107**:8369-8374 http://dx.doi.org/10.1073/pnas.0909951107.

52. Chi J, Wang F, Li L, Feng D, Qin J, Xie F, Zhou F, Chen Y, Wang J, Yao K: **The role of MAPK in CD4(+) T cells toll-like receptor 9-mediated signaling following HHV-6 infection**. *Virology* 2012, **422**:92-98 http://dx.doi.org/10.1016/j.virol.2011.09.026.

53. Murakami Y, Tanimoto K, Fujiwara H, An J, Suemori K, Ochi T, Hasegawa H, Yasukawa M: **Human herpesvirus 6 infection impairs Toll-like receptor signaling**. *Virol J* 2010:91 http://dx.doi.org/10.1186/1743-422X-7-91.

54. Hu X, Yu J, Crosby SD, Storch GA: **Gene expression profiles in febrile children with defined viral and bacterial infection**. *Proc Natl Acad Sci U S A* 2013:12792-12797 http://dx.doi.org/10.1073/pnas.1302968110.

55. Kawamura Y, Yamazaki Y, Ohashi M, Ihira M, Yoshikawa T: **Cytokine and chemokine responses in the blood and cerebrospinal fluid of patients with human herpesvirus 6B-associated acute encephalopathy with biphasic seizures and late reduced diffusion**. *J Med Virol* 2014, **86**:512-518 http://dx.doi.org/10.1002/jmv.23788.

56. Kawamura Y, Nakai H, Sugata K, Asano Y, Yoshikawa T: **Serum biomarker kinetics with three different courses of HHV-6B encephalitis**. *Brain Dev* 2013, **35**:590-595 http://dx.doi.org/10.1016/j.braindev.2012.08.005.

57. Kawamura Y, Sugata K, Ihira M, Mihara T, Mutoh T, Asano Y, Yoshikawa T: **Different characteristics of human herpesvirus 6 encephalitis between primary infection and viral reactivation**. *J Clin Virol* 2011, **51**:12-19 http://dx.doi.org/10.1016/j.jcv.2011.02.002.

58. Tanner A, Carlson SA, Nukui M, Murphy EA, Berges BK: **Human herpesvirus 6A infection and immunopathogenesis in humanized Rag2$^{-/-}$ γc$^{-/-}$ mice**. *J Virol* 2013, **87**:12020-12028 http://dx.doi.org/10.1128/JVI.01556-13.

59. Nordström I, Eriksson K: **HHV-6B induces IFN-lambda1 responses in cord plasmacytoid dendritic cells through TLR9**. *PLoS ONE* 2012, **7**:e38683 http://dx.doi.org/10.1371/journal.pone.0038683.

60. Kittaka S, Hasegawa S, Ito Y, Ohbuchi N, Suzuki E, Kawano S, Aoki Y, Nakatsuka K, Kudo K, Wakiguchi H *et al.*: **Serum levels of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinases-1 in human herpesvirus-6-infected infants with or without febrile seizures**. *J Infect Chemother* 2014 http://dx.doi.org/10.1016/j.jiac.2014.07.017.

61. Uno H, Kabashima K, Tohyama M, Watanabe T, Hashimoto K, Iijima M, Sueki H, Watanabe H: **TNF-α as a useful predictor of human herpesvirus-6 reactivation and indicator of the disease process in drug-induced hypersensitivity syndrome (DIHS)/drug reaction with eosinophilia and systemic symptoms (DRESS)**. *J Dermatol Sci* 2014, **74**:177-179.

62. Ishida T, Kano Y, Mizukawa Y, Shiohara T: **The dynamics of herpesvirus reactivations during and after severe drug eruptions: their relation to the clinical phenotype and therapeutic outcome**. *Allergy* 2014, **69**:798-805 http://dx.doi.org/10.1111/all.12410.

63. Isegawa Y, Mukai T, Nakano K, Kagawa M, Chen J, Mori Y, Sunagawa T, Kawanishi K, Sashihara J, Hata A *et al.*: **Comparison of the complete DNA sequences of human herpesvirus 6 variants A and B**. *J Virol* 1999, **73**:8053-8063.

64. Gompels UA, Nicholas J, Lawrence G, Jones M, Thomson BJ, Martin ME, Efstathiou S, Craxton M, Macaulay HA: **The DNA sequence of human herpesvirus-6: structure, coding content, and genome evolution**. *Virology* 1995, **209**:29-51 http://dx.doi.org/10.1006/viro.1995.1228.

65. Megaw AG, Rapaport D, Avidor B, Frenkel N, Davison AJ: **The DNA sequence of the RK strain of human herpesvirus 7**. *Virology* 1998, **244**:119-132 http://dx.doi.org/10.1006/viro.1998.9105.

66. Nicholas J: **Determination and analysis of the complete nucleotide sequence of human herpesvirus 7**. *J Virol* 1996, **70**:5975-5989.

67. Øster B, Höllsberg P: **Viral gene expression patterns in human herpesvirus 6B-infected T cells**. *J Virol* 2002, **76**:7578-7586.

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Current Opinion in Virology

Roseoloviruses and their modulation of host defenses

Amy W Hudson

Human cytomegalovirus (HCMV), the prototypical human β-herpesvirus, encodes approximately 40 known gene products that function to subvert our host defense mechanisms. From HCMV, we have learned about interferon signaling, cytokine function, chemokine signaling, natural killer (NK) cells' cytotoxicity toward tumors and virus-infected cells, antigen processing and presentation, and protective initiation of the apoptotic signaling cascade. With each successive discovery of novel host evasion mechanism encoded by the cytomegaloviruses, we illuminate what these herpesviruses have learned over the course of their 100 MYr-long evolution with their hosts. As much as we have learned from HCMV, the other members of the human β-herpesvirus family, HHV-6 and HHV-7, are closely-related and yet largely unexplored. These viruses likely have much yet to teach us.

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Introduction

To achieve optimal reproduction and spread, viruses realign host cellular processes to create a more hospitable environment. Over the course of their co-evolution, viruses have pushed their hosts to develop and fortify an arsenal of sophisticated defense mechanisms. Mammalian hosts, for example, are able to immediately sense the introduction of foreign viral products. Recognition of these viral products provokes rapid upregulation of host innate immune response genes, including soluble cytokines and chemokines, which together influence almost every other aspect of the host response to pathogens. Shortly after cytokine release and signaling, host natural killer (NK) cells are activated to recognize and destroy virus-infected cells. The adaptive immune response then ensues, usually eliminating the virus-infected cells with cytotoxic T cells and neutralizing antibodies. If all else fails, individual infected host cells are programmed to undergo selfless sacrifice – apoptosis for the greater good.

The intimate relationships that occur between hosts and viruses that establish long-lived, latent, or persistent infections have further pushed the evolution of the host defense network. Herpesviruses, for example, after primary lytic infection, remain latent or persistent within the host throughout the life of the host. In so doing, they must necessarily interact with and evade host defense mechanisms. It is therefore not surprising that herpesviruses devote as much as half of their large (~125-240 kB) genomes to counteracting host defenses.

Here, we illustrate the individual cunning of the β-herpesviruses. Human cytomegalovirus (HCMV), one of the most stealthy, successful, and well-studied human β-herpesviruses, is an example of a virus that has fought - and seems to be winning - a long evolutionary battle to live, propagate and disseminate in the face of extensive and sophisticated defense mechanisms. But HCMV is not the only β-herpesvirus that seems to be winning this battle. Human herpesviruses-6A, -6B and -7 are arguably equally as ''successful'' as HCMV. While HCMV infects 50-80% of the US population by age 40, HHV-6A, HHV-6B, and HHV-7 infect over 90% of the population before the age of 6 [1,2]. Like HCMV, HHV-6A, -6B, and -7 also remain latent or persistent throughout the life of their hosts. HCMV, HHV-6A and -6B, and HHV-7 share a core set of essential β-herpesvirus genes involved in DNA replication, packaging, and encapsidation. The other, ''non-essential'' genes in the β-herpesvirus genomes are largely devoted to escaping host defenses. Indeed, our current understanding of host defense mechanisms is derived in part from the what we have learned from HCMV, and distantly related murine CMV. Study of these viruses has shed light upon interferon signaling, cytokine function, chemokine signaling, NK cytotoxicity toward tumors and virus-infected cells, antigen processing and presentation, and protective initiation of the apoptotic signaling cascade. With each discovery of novel host evasion mechanism encoded by cytomegaloviruses, we illuminate what these herpesviruses have learned over their 100 MYr-long evolution with their hosts. As much as we have learned from HCMV, the closely-related and largely unexplored HHV-6A,

HHV-6B, and HHV-7 would seem to have much still to teach us.

The host interferon response

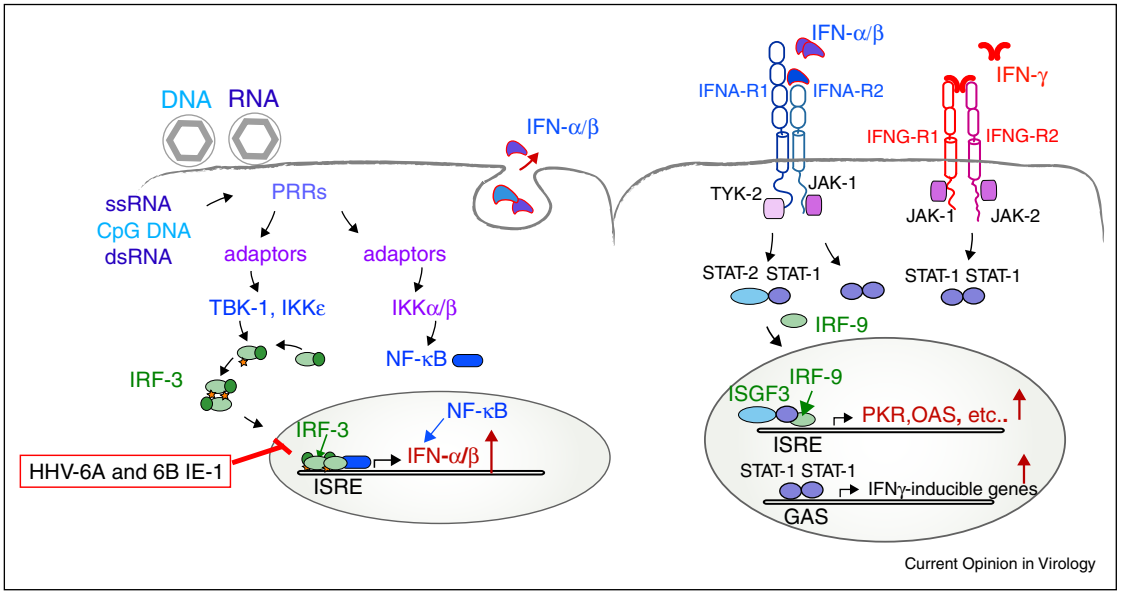
After recognition of uniquely foreign viral products such as dsRNA or cytosolic DNA by pattern recognition receptors, host signaling cascades lead to the IκB kinase- NFκB-, and IRF3/7/9-induced transcription of type I interferons (IFNα and IFNβ) (See Figure 1). Type I interferons signal through IFN receptors, JAK/STAT adaptor kinases, and ultimately use the STAT1/STAT2/IRF9 complex to induce transcription of myriad interferon-inducible genes. These interferon-responsive gene products, which include protein kinase R (PKR) and 2'-5' oligoadenylate synthase (OAS), induce an anti-viral state in the host, preventing viruses from usurping cellular protein synthesis machinery for the production of viral proteins. Type II interferon (IFN-γ), or ''immune'' interferon, is released by immune cells in response to cytokines. IFN-γ then stimulates the launch of an effective adaptive immune response, activating T and B lymphocytes.

To minimize the inhospitable environment they encounter upon entering the host cell, viruses encode multiple means of quelling the innate immune response. HCMV, for example, encodes 7 protein products that hamper the host interferon response (Table 1). HHV-6A and -6B have also been shown to impair interferon signaling: Jaworska, et al. have shown that the **HHV-6A and -6B IE-1** proteins may either prevent or disrupt the dimerization of IRF3, reducing the presence of IRF3 in the nucleus, and reducing transcription of IRF3-inducible genes downstream of IFNβ signaling [3••] (Figure 1).

Cytokine and chemokine signaling

Cellular proinflammatory cytokines IL-1β and TNFα participate in the host defense against viruses through recruitment of inflammatory cells and activate signaling cascades involved in both the innate and adaptive immune response. TNFα is secreted by activated macrophages, and binds to TNF receptors (TNFR) expressed on most tissues (for review, see [4]). TNFR signaling activates NFκB, and can induce fever, apoptosis, and inflammation, thus viruses benefit from developing means to downregulate the functions of inflammatory cytokines like TNFα and IL-1β. Lymphocyte trafficking to sites of infection depends upon the local presence of chemokines, chemoattractant cytokines which attract immune cells and play a role in the activation of their effector mechanisms.

Figure 1



The interferon response. Viral products (e.g.,single-stranded RNA, CpG DNA, or dsRNA) are sensed by pattern recognition receptors (e.g.,TLRs, RIG-I, IFI16, mda5) and a signaling cascade ensues, involving adaptor proteins, ultimately leading to interferon regulatory factor-3 (IRF-3) phosphorylation, which allows dimerization, translocation to the nucleus, and, with βcatenin and p300, binding to the interferon-stimulated response element (ISRE) to upregulate transcription of the type I interferons IFNα/β. IFNα or β is secreted and binds to IFNA-Receptors on neighboring cells, inducing another signaliing cascade mediated by JAK-1, TYK-2, and STATs. STAT-1, STAT-2, and IRF-9 comprise the interferon-stimulated gene factor-3 (ISGF3), which bind to ISRE and upregulate type I IFN-inducible genes, such as PKR and OAS. Also shown is the Type II IFN signaling pathway, induced by IFN-γ, mediated by JAK-1, -2, and STAT-1 homodimers. Type II IFN signaling results in upregulation of genes possessing an interferon-gamma activating sequence (GAS) element. HHV-6A and -6B IE-1 proteins prevent the dimerization of IRF-3, inhibiting interferon signaling.

Table 1

Listing of HCMV gene products and their function in evasion of host defenses. Note that only 4 of the HCMV genes possess positional homologs in the roseoloviruses (italic), suggesting that the roseolovirus gene products that participate in evasion of host defenses are likely to be unique to the roseoloviruses. ORFs possessing positional homologs in HHV-6 are in italics.

ORF	Function	Positional homolog in HHV6	References
	IFN response		
UL83	*Impairs localization of interferon regulatory factors*	*U54*	*[43,44]*
UL83	Inhibits IFI16-mediated DNA sensing		[45]
UL123 (IE1)	Interferes with Type I and Type II IFN signaling; sequesters STAT2		[46,47]
UL122 (IE2)			[48,49]
TRS1	Bind to dsRNA and prevent PKR-induced shutoff of protein synthesis		[50–53]
IRS1	Bind to dsRNA and prevent PKR-induced shutoff of protein synthesis		[50–53]
ORF94	Reduces OAS expression and impairs OAS function		[54]
	Cytokine response		
UL144			[55,56]
UL144	Binds to BTLA, mimicking the function of HVEM, inhibiting T cell proliferation		[57]
UL111a			[58,59]
UL21.5	Soluble CC chemokine receptor that functions as a decoy to modulate host response		[60]
US27	7-tm GPCR homolog; no ligand found, function unknown		[61,62]
US28	7-tm GPCR homolog; signals constitutively in response to multiple CC chemokines		[62–66]
UL33	*7-tm GPCR homolog; heteroligomerizes with US28 and regulates CCR5 and CXCR4*	*U12*	*[62,67]*
UL78	*7-tm GPCR homolog; heteroligomerizes with US28 and regulates CCR5 and CXCR4*	*U51*	*[62]*
UL146			[68]
UL147			[69]
UL128			[70,71]
	Class I MHC antigen presentation		
US2	Binds to class I MHC molecules and results in retrotranslocation and degradation		[72]
US11	Binds to class I MHC molecules and results in retrotranslocation and degradation		[73]
US3	Binds to class I MHC molecules and impedes trafficking from the ER to the Golgi		[74–77]
US6	Blocks the TAP transporter, impeding peptide entry into the ER, affecting stability of class I		[78,79]
	NK response		
UL16	Binds to ULBP1, ULBP2, ULBP6, and MICB and downregulates these NK activating ligands		[28,29,33–36]
US18	Induce the lysosomal degradation of MICA		[80]
US20	Induce the lysosomal degradation of MICA		[80]
UL142	Retains MICA in the Golgi		[81,82]
UL141	Sequesters CD155 and CD112, activating ligands for DNAM-1 or CD96		[83,84]
miR-UL112	miRNA that targets and downregulates MICB		[85]
UL18 Class I	homolog; acts as a decoy NK-inhibitory receptor; also binds LIR-1 inhibitory receptor		[86,87]
UL83	Binds to NKp30 NK activating receptor and suppresses signaling		[88]
	Apoptosis		
UL123 (IE1)	Activates Akt, which acts on IkK to release NFkB to activate tx of anti-apoptotic genes		[89]
UL122 (IE2)	Inhibits NFkB's DNA-binding activity		[48,49,90]
UL141	Binds to TRAIL receptors,retaining them, reducing TRAIL-dependent NK-mediated killing		[91]
UL36	A class I MHC homolog that is a viral inhibitor of caspase-8-induced apoptosis (vMIA)		[92,93]
UL37	Binds to Bax and prevents Bax from arriving in the mitochondria, reducing its activity		[94,95]
UL38	*Prevents apoptosis; mechanism unknown*	*U19*	*[96,97]*
UL28	Induces caspase-dependent apoptosis by activating caspases 8 and 10, independent of TNFR		[98]
miR-UL70-3p	Silences pro-apoptotic genes MOAP1, PHAP, and ERN1		[99]
miR-UL148D	Silences pro-apoptotic genes MOAP1, PHAP, and ERN1		[99]

To minimize cytokine effects, viruses encode multiple means of usurping chemokine and cytokine signaling pathways. HCMV, for example, encodes 12 protein products that may affect chemokine signaling (Table 1). Eight of these HCMV genes encode G-protein coupled receptors or chemokines, as predicted from their sequence homology. HHV-6A, HHV-6B, and HHV-7 possess positional and structural homologs of two of these HCMV genes, UL33 and UL78. **HHV-6B** and **HHV-7 U12** and **U51** gene products are positional and structural homologs of HCMV UL33 and UL78, respectively. Both **HHV-7 U12 and U51** have been shown to be functional chemokine receptors that act as ligands for CCL22 and CCL19 [5••]. **HHV-7** U21 and U51 share ~66% and 50% homology with their HHV-6A and -6B GPCR counterparts, while HHV-6A and HHV-6B chemokine receptors are 94% identical. HHV-6B U12 was shown to be a functional chemokine receptor for CCL2, CCL4, and

CCL5 [6]. HHV-6A U51A signals constitutively and also inducibly responds to CCL2, CCL5, CCL7, CCL11, and CCL13 [7,8].

HHV-6B U83 is a secreted β-chemokine shown to have monospecific β-chemotactic activity for CCR2 [9••,10••]. The **HHV-6A** homolog of **U83** (U83A) shares 86% identity with **HHV-6B U83**, and has β-chemokine activity for CCR1, CCR4, CCR5, CCR6, and CCR8 [11••]. Catusse, et al. have shown that U83A can functionally bind to CCR5 with higher affinity than human chemokines, displacing their binding, inhibiting chemotaxis of human leukocytes, and inhibiting infection by HIV-1 strains that use CCR5 as a co-receptor [12]. The functionality of these HHV-6 gene products in the context of HHV-6 infection has not yet been investigated, largely due to the historical lack of a genetically manipulable BAC system for HHV-6. Interestingly, HHV-7 does not contain a U83 homolog.

T cell activation

The T cell response is critical in the adaptive immune response to virus infection. Since HHV-6A, HHV-6B, and HHV-7 predominantly infect $CD4^+$ T-cells, an activated T cell response directed against HHV-6A, -6B and -7-infected cells might be complicated by infection of the T cells themselves. HHV-6A and HHV-6B encode two proteins that could affect T-cell proliferation during HHV-6A or -6B infection. The **HHV-6B U54** gene product, for example, was recently shown to inhibit IL-2 gene expression [13••]. IL-2 is necessary for growth and proliferation of T cells as they differentiate during the adaptive immune response. Iampietro et al., found that the U54-encoded tegument protein interacts with the phosphatase calcineurin to prevent the dephosphorylation of NFAT, which blocks its nuclear translocation [13••]. Nuclear translocation of NFAT is necessary for its activation of NFAT-inducible genes, which include IL-2. Interestingly, despite 80% amino acid identity, **HHV-6A U54** does not inhibit IL-2 gene expression [13••]. The function of **HHV-7 U54**, which shares only 44% identity with HHV-6B U54, has not been investigated. HHV-6A, -B, and -7 U54 are the positional homologs of HCMV UL82/83.

HHV-6A U24 encodes a unique tail-anchored protein that downregulates the CD3 T cell receptor signaling complex from the cell surface [14••,15••]. The physiological benefit of downregulating CD3 during HHV-6A infection is unclear, but Sullivan and Coscoy suggest three possibilities: 1) U24 expression might prevent T cell activation, which would, in turn, reduce the release of cytokines and potentially dampen the adaptive immune response. 2) reducing surface expression of the T cell receptor in infected cells might prevent reactivation, helping to maintain a latent state. Expression profiling analysis suggests that U24 is an early gene product [16], but further experimentation is necessary to ascertain whether U24 maintains expression during latency. 3) Sullivan and Coscoy note that pretreatment of cells with an anti-CD3 antibody enhances HHV-6 replication, thus in downregulating CD3, perhaps U24 reduces HHV-6A titers so that they do not induce large-scale immune activation [17]. With the availability of the BAC genetic system for manipulation of the HHV-6A genome, future investigation aimed at elucidating the physiological benefit of CD3 downregulation to HHV-6A may to illuminate the novel pathophysiological features of HHV-6 infection [18]. **HHV-6B U24** is quite similar to **HHV-6A U24**, but **HHV-7 U24** shares only 30% identity with HHV-6A U24 [19]. **HHV-6A and HHV-7 U24** remain uncharacterized.

Antigen processing and presentation to cytotoxic T cells

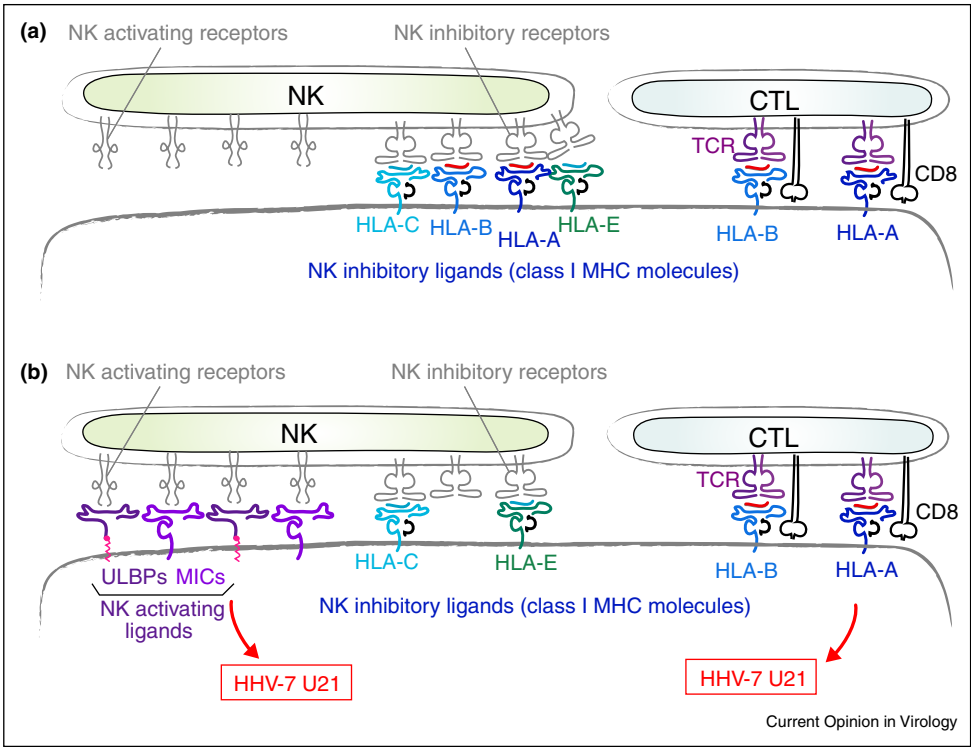
To identify virus-infected cells, class I MHC molecules present peptide antigens derived from intracellular proteins – host and viral antigens alike – for scrutiny by cytotoxic T cells. Peptides generated in the cytosol are transported into the ER via TAP transporters, and are loaded onto newly-assembled class I MHC molecules (in humans, termed HLA-A, HLA-B and HLA-C) in the ER. Once assembled, the peptide-bound class I molecules travel *via* the secretory pathway to the plasma membrane, where they present self- or virus-derived peptides to cytotoxic T lymphocytes.

NK cell function

Before an adaptive cytotoxic T cell response can develop, NK cells participate in the innate immune response against virus infection. NK cells recognize and kill virus-infected cells and tumor cells through recognition of NK *activating* ligands. NK activating ligands are not expressed constitutively, but instead are induced by virus infection or in response to cell stressors (e.g. DNA damage) (See Figure 2). The NK cell integrates the activating and inhibitory signals it receives in formulating a decision to kill its target. Class I MHC molecules are *inhibitory ligands* for NK cells, thus the function of the four different HCMV gene products in downregulating class I molecules (Table 1), would seemingly serve to enhance NK cytotoxicity toward virus-infected cells. Strategically, however, several of these viral proteins have been shown to downregulate some HLA alleles (HLA-A and HLA-B) and not others (e.g. HLA-C) [20,21], leaving some class I alleles remaining on the cell surface to act as NK inhibitory ligands. This tactic is practiced by other viruses as well; HIV Nef and HHV-8 K3 proteins are also selective for HLA-A and HLA-B alleles [22,23].

None of the four HCMV gene products that affect class I MHC molecules possess positional homologs in the roseoloviruses. However, HHV-7 encodes a unique

Figure 2



T- and NK-cell recognition of virus-infected cells. (a) Classical class I MHC molecules, (blue, HLA-A, HLA-B, and HLA-C) present peptides to CD8+ T cells. T cell receptors (TCR, purple), with a co-receptor (CD8, black), can recognize peptides presented in the context of class I molecules and secrete perforin and granzymes to kill a target cell. Both classical (blue) and non-classical class I MHC molecules (green, HLA-E, e.g.) can act as inhibitory ligands for NK cell receptors. (b) When a virus infects a cell, NK activating ligands (purple, ULBPs, MICs) are upregulated. NK cells integrate the inhibitory and activating signals they receive. If activating signals predominate, NK cells can secrete perforin and granzymes to kill a target cell. HHV-7 U21 downregulates classical and non-classical class I molecules, as well as NK activating ligands, presumably escaping both T- and NK-cytotoxicity.

protein that reduces cell surface expression of class I MHC molecules. The **HHV-7 U21** gene product binds to and reroutes class I MHC molecules to lysosomes [24••,25••]. Interestingly, U21 can associate with and reroutes all class I gene products, including HLA-A, HLA-B, HLA-C, as well as the non-classical class I molecules HLA-E and HLA-G. U21 can even reroute the murine class I molecule H-2K^{b} [26]. Given the ability of U21 to reduce surface expression of NK-inhibitory class I MHC molecules, we surmised that HHV-7 must encode other novel means of preventing NK activation.

In addition to the selective downregulation of NK-inhibitory class I MHC molecules, another viral strategy to escape NK engagement involves downregulation of NK-*activating* ligands from the cell surface (Figure 2). Cellular NK-activating ligands were first discovered when investigators queried the binding partners of the HCMV UL16 gene product [27] (Table 1). UL16 was found to bind to two members of a family of cellular proteins termed UL16-binding proteins, or ULBPs, sequestering them in the ER [28,29]. UL16 was also found to associate with MICB (MHC class I chain-related protein B) [29]. Both MICs and ULBPs share structural similarity with class I MHC molecules [30–32]. HMCV UL16 binds to ULBP1, ULBP2, ULBP6, and MICB, and traps these activating ligands intracellularly, reducing NK engagement of HCMV-infected cells [33–36].

The same **HHV-7** gene product that binds to and downregulates class I MHC molecules, **HHV-7 U21,** also downregulates NK activating ligands MICA, MICB, and reroutes ULBP1 to lysosomes [37••], thus U21 appears to have dual-function, downregulating class I MHC molecules as well as NK activating ligands. While HCMV encodes 7 proteins and a miRNA that are devoted to downregulating the NK response, only U21 has been identified to affect NK function in HHV-7. It is not known how HHV-7 (or HHV-6) has evolved to cope with the other NK activating ligands CD155, CD112, ULBP2-6, or NKp30, all of which are targets of HCMV proteins (Table 1). **HHV-6A and HHV-6B U21**, which share only ~30% identity with

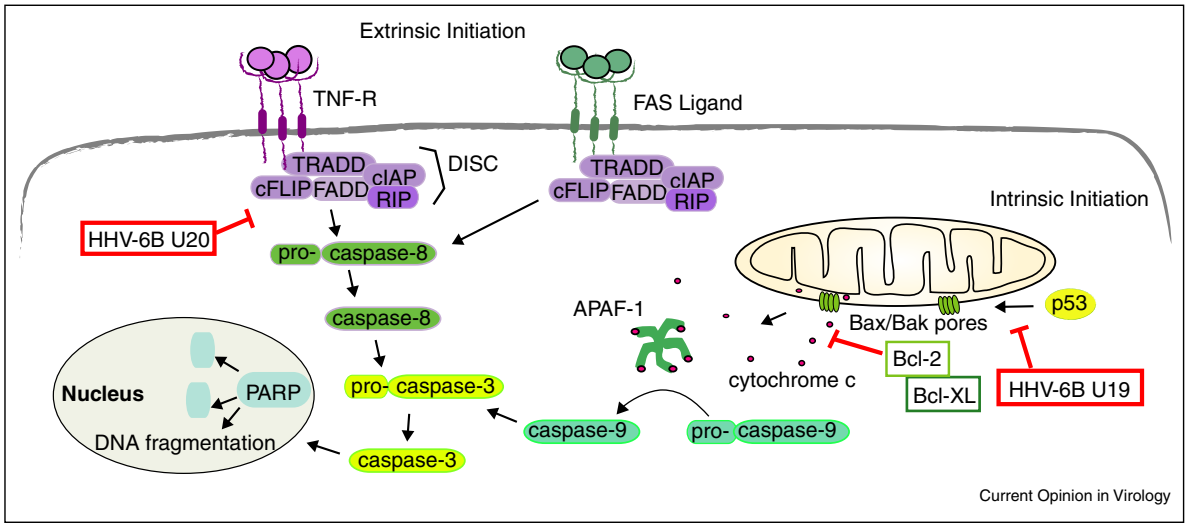
HHV-7 U21, can also bind to and reroute class I MHC molecules to the lysosomal compartment, but the affinity of HHV-6A and -6B U21 for class I MHC molecules is considerably weaker; it is therefore possible that HHV-6 U21 molecules assume an entirely different function [38••]. If so, one would assume that HHV-6A and -6B must encode other means of reducing NK cytotoxicity toward HHV-6-infected cells.

Apoptosis

The host response can also include the induction of apoptosis, as a means to prevent the virus from spreading. The signal to initiate apoptosis can come from within the infected cell, initiated by infection-induced stimuli such as DNA damage or other infection-induced cell stress, or the signal can come from the external environment, in the form of TNF-family binding to cell surface TNF'death' receptors (See Figure 3). Host cell-intrinsic stimuli are sensed by BH3-only domain-containing proteins of the Bcl-2 family such as **Bim** and **Bad**. Activation of these proteins result in insertion of proapoptotic Bcl-2 family members such as pore-forming **Bax** and **Bak**, into the mitochondrial membrane (for review, see [39]). Release of cytochrome c from mitochondria into the cytosol activates **caspase-9** and results in formation of a complex of **APAF-1, cytochrome c**, and **caspase-9**, called the apoptosome, which mediates cleavage of downstream **caspases-3 and -7**, which in turn induce the effects of apoptosis, a result of degradation of many downstream cellular proteins involved in DNA repair and cell maintenance. Host-cell-extrinsic stimuli, such as TNF-family cytokine members TNFα, Fas ligand, and TRAIL, induce apoptosis through a death-induced signaling complex (DISC) assembled on the TNF-receptor family of death domain containing receptors. Death-domain-containing adaptors such as fas-associated death domain (**FADD**) and TNFR-associated death domain (**TRADD**) mediate activation of **caspase-8**, which can then activate **caspases-3 and -7**, converging upon the same effectors as the intrinsic pathway. Initiation of the apoptotic cascade results in dire consequences to the cell, and is therefore tightly regulated by anti-apoptotic members of the BH3-domain family, such as **Bcl-2** and **Bcl-X_L**, or mitochondrial inhibitors of apoptosis (**MIAs**)(for review see [40]). Thus, for a virus to inhibit the apoptotic program, it would either need to upregulate anti-apoptotic proteins, or inhibit the pro-apoptotic cascade. Not surprisingly, HCMV encodes at least 4 proteins and 2 miRNAs that possess these qualities.

Like HCMV, **HHV-6B** manipulates both the extrinsic and intrinsic apoptotic cascades: **HHV-6B U20** expression impairs PARP cleavage and cleavage of caspase-3 and caspase-8, preventing extrinsically-induced apoptosis through an unknown mechanism [41••]. **HHV-6B U19**, the positional homolog of HCMV **UL38**, was recently shown to impair intrinsic, p53-mediated apoptosis [42••].

Figure 3



The extrinsic and intrinsic apoptotic cascades. Extrinsic signals, such as TNFα and Fas signal through trimeric TNF Receptors or TNFR-like receptors through a death-inducing signaling complex (DISC) to cleave and activate caspases, beginning with caspase-8, and ultimately converging upon activation of effector caspases and nucleases. The intrinsic pathway is initiated in the mitochondria, which integrate intracellular apoptotic signals (e.g., DNA damage, ER stress), inducing permeabilization of the mitochondria by Bax/Bak channels. Mitochondrial cytochrome c, when in the cytosol, forms an'apoptosome' with APAF-1, which activates caspase cleavage to induce DNA fragmentation and cell death. HHV-6B U19 and U20 prevent both extrinsic and intrinsic apoptosis.

Table 2

Listing of HHV-6A, HHV-6B, and HHV-7 gene products and their function in evasion of host defenses. ORFs possessing positional homologs in HCMV are in italics.

ORF	Function	HCMV ORF	References
	IFN response		
6A, 6B IE-1	Prevent or disrupt the dimerization of IRF3, reducing transcription of IRF3-inducible genes		[3••]
	Cytokine response		
7 U12	*Positional and structural homolog of UL33*	*UL33*	*[5••]*
7 U51	*Positional and structural homolog of UL78*	*UL78*	*[5••]*
6A U83	β-Chemokine having chemotactic activity for CCR2		[9,10]
6B U83	Chemotactic activity for CCR2, CCR4, CCR5, CCR6, and CCR8		[11]
	Class I MHC antigen presentation		
7 U21	Binds to class I MHC molecules and reroutes them to lysosomes		[20,21,26]
	NK response		
7 U21	Binds to NK activating ligands and reroutes them to lysosomes		[37]
	Apoptosis		
6B U19	*Impairs intrinsic, p53-mediated apoptosis; mechanism unknown UL38*		*[42]*
6B U20	Impairs extrinsic PARP cleavage and cleavage of caspase-3 and -8; mechanism unknown		[41]
	T cell response		
6A U24	Downregulates CD3 T cell receptor		[16,17]
6B U54	*Impairs expression of IL-2*	*UL82/83*	*[13]*

HHV-7 U20 shares only 20% identity with **HHV-6B U20**, and does not seem to affect PARP cleavage (S. Konrad and A. Hudson, unpublished results), and **HHV-7 U19** remains uncharacterized.

Summary

Given the similarity of β-herpesvirus family members HHV-6A, HHV-6B, and HHV-7 to the prototypical β-herpesvirus HCMV, and the sheer number of HCMV proteins (~39) that have been described to escape the host arsenal of defense, the 3 proteins from HHV-7 (U12, U51, and U21) and the 6 proteins from HHV6A or HHV6B (IE1, U19, U20, U24, U54, and U83) seem paltry in comparison (Table 2). With the arsenal of proteins encoded by HCMV found to impair the host response, one wonders how, with so few means of doing so themselves, HHV-6A, -6B, and -7 are able to survive in the face of host defenses? Is the lifestyle of the roseoloviruses so different from HCMV that they do not require such an arsenal of host evasion proteins? Do the roseoloviruses encode fewer host evasion proteins because the few that they encode are far more effective than those encoded by HCMV? Or do the roseoloviruses encode many more, as yet unidentified, means of escape? It seems likely that some additional gene products from HHV-6A, HHV-6B, and HHV-7 are also involved in host evasion, with as-yet undiscovered mechanisms remaining to be elucidated.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hall CB, Long CE, Schnabel KC, Caserta MT, McIntyre KM, Costanzo MA, Knott A, Dewhurst S, Insel RA, Epstein LG: **Human herpesvirus-6 infection in children. A prospective study of complications and reactivation**. *N Engl J Med* 1994, **331**:432-438 PMID: 8035839.

2. Huang L-M, Lee C-Y, Liu M-Y, Lee P-I: **Primary infections of human herpesvirus-7 and herpesvirus-6: a comparative, longitudinal study up to 6 years of age**. *Acta paediatrica (Oslo, Norway: 1992)* 1997, **86**:604-608 PMID: 9202795.

3. •• Jaworska J, Gravel A, Fink K, Grandvaux N, Flamand L: **Inhibition of transcription of the beta interferon gene by the human herpesvirus 6 immediate-early 1 protein**. *Journal of virology* 2007, **81**:5737-5748 PMID: 17376932..
This study shows that the HHV-6 IE-1 protein suppresses type- I IFN induction.

4. Naudé PJW, den Boer JA, Luiten PGM, Eisel ULM: **Tumor necrosis factor receptor cross-talk**. *The FEBS journal* 2011, **278**:888-898 PMID: 21232019.

5. •• Tadagaki K, Yamanishi K, Mori Y: **Reciprocal roles of cellular chemokine receptors and human herpesvirus 7-encoded chemokine receptors, U12 and U51**. *J Gen Virol* 2007, **88**:1423-1428 PMID: 17412969..
Here, the authors expressed HHV-7 U12 and U51 in cells and found that like human chemokine receptors CCR4 and CCR7, U12 and U51 respond to CCL17, CCL19, CCL21, and CCL22.

6. Isegawa Y, Ping Z, Nakano K, Sugimoto N, Yamanishi K: **Human herpesvirus 6 open reading frame U12 encodes a functional β-chemokine receptor**. *J Virology* 1998, **72**:6104-6112 PMID: 15806130.

7. Catusse J, Spinks J, Mattick C, Dyer A, Laing K, Fitzsimons C, Smit MJ, Gompels UA: **Immunomodulation by herpesvirus U51A chemokine receptorvia CCL5 and FOG-2 down-regulation plus XCR1 and CCR7 mimicry in human leukocytes**. *Eur J Immunol* 2008, **38**:763-777 PMID:.

8. Milne RS, Mattick C, Nicholson L, Devaraj P, Alcami A, Gompels UA: **RANTES binding and down-regulation by a novel human herpesvirus-6 β chemokine receptor**. *J Immunol* 2000, **164**:2396-2404 PMID: 57732081.

9. •• Lüttichau HR, Clark-Lewis I, Jensen PØ, Moser C, Gerstoft J, Schwartz TW: **A highly selective CCR2 chemokine agonist encoded by human herpesvirus 6**. *J Biol Chem* 2003, **278**:10928-10933 PMID: 12554737..

This study shows that the U83 ORF from HHV-6B encodes a CC chemokine that functions as a selective agonist for CCR2.

10. •• Clark DJ, Catusse J, Stacey A, Borrow P, Gompels UA: **Activation of CCR2+ human proinflammatory monocytes by human herpesvirus-6B chemokine N-terminal peptide**. *J Gen Virol* 2013, **94**:1624-1635 PMID: 23535574..

This study provides evidence of a role for HHV-6B U54 in inhibition of IL-2 gene expression. Surprisingly, despite 80% identity, HHV-6A U54 does not inhibit the IL-2 promoter.

11. •• Dewin DR, Catusse J, Gompels, UA:: **Identification and Characterization of U83A Viral Chemokine, a Broad and Potent -Chemokine Agonist for Human CCRs with Unique Selectivity and Inhibition by Spliced Isoform**. *J Immunol* 2005, **176**:544-556.

This study demonstrates that, in contrast to HHV-6B U83, HHV-6A U83 has potent ß-chemokine activity for CCR1, CCR4, CCR5, CCR6, and CCR8

12. Catusse J, Parry CM, Dewin DR, Gompels UA: **Inhibition of HIV-1 infection by viral chemokine U83A via high-affinity CCR5 interactions that block human chemokine-induced leukocyte chemotaxis and receptor internalization**. *Blood* 2007, **109**:3633-3639 PMID: 17209056.

13. •• Iampietro M, Morissette G, Gravel A, Flamand L: **Inhibition of Interleukin-2 Gene Expression by Human Herpesvirus 6B U54 Tegument Protein**. *J Virol* 2014. PMID: 25122797..

This study provides evidence of a role for HHV-6B U54 in inhibition of IL-2 gene expression. Surprisingly, despite 80% identity, HHV-6A U54 does not inhibit the IL-2 promoter.

14. •• Sullivan BM, Coscoy L: **Downregulation of the T-cell receptor complex and impairment of T-cell activation by human herpesvirus 6 u24 protein**. *J Virol* 2008, **82**:602-608 PMID: 17977973..

The authors of this paper and the next (12) provide evidence that HHV-6A U24 is a tail anchored membrane protein that downregulates the T cell receptor CD3.

15. •• Sullivan BM, Coscoy L: **The U24 protein from human herpesvirus 6 and 7 affects endocytic recycling**. *J Virol* 2010, **84**:1265-1275 PMID: 19923186.

16. Yao K, Mandel M, Akyani N, Maynard K, Sengamalay N, Fotheringham J, Ghedin E, Kashanchi F, Jacobson S:: **Differential HHV-6A gene expression in T cells and primary human astrocytes based on multi-virus array analysis**. *Glia* 2006, **53**:789-798 PMID: 16541415.

17. Roffman E, Frenkel N:: **Replication of human herpesvirus-6 in thymocytes activated by anti-CD3 antibody**. *J Infect Dis* 1991, **164**:617-618 PMID: 1651362.

18. Tang H, Kawabata A, Yoshida M, Oyaizu H, Maeki T, Yamanishi K, Mori Y: **Human herpesvirus 6 encoded glycoprotein Q1 gene is essential for virus growth**. *Virology* 2010, **407**:360-367 PMID:.

19. Dominguez G, Dambaugh T, Stamey F, Dewhurst S, Inoue N, Pellett PE: **Human herpesvirus 6B genome sequence: coding content and comparison with human herpesvirus 6A**. *J Virol* 1999, **73**:8040-8052 PMID: 10482553.

20. Llano M, Guma M, Ortega M, Angulo A, Lopez-Botet M: **Differential effects of US2, US6 and US11 human cytomegalovirus proteins on HLA class Ia and HLA-E expression: impact on target susceptibility to NK cell subsets**. *Eur J Immunol* 2003, **33**:2744-2754 PMID:.

21. Gewurz BE, Wang EW, Tortorella D, Schust DJ, Ploegh HL: **Human cytomegalovirus US2 endoplasmic reticulum-lumenal domain dictates association with major histocompatibility complex class I in a locus-specific manner**. *J Virol* 2001, **75**:5197-5204 PMID:.

22. Ishido S, Wang C, Lee BS, Cohen GB, Jung JU: **Downregulation of major histocompatibility complex class I molecules by Kaposi's sarcoma-associated herpesvirus K3 and K5 proteins**. *J Virol* 2000, **74**:5300-5309 PMID: 10799607.

23. Cohen GB, Gandhi RT, Davis DM, Mandelboim O, Chen BK, Strominger JL, Baltimore D: **The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells**. *Immunity* 1999, **10**:661-671 PMID:.

24. •• Hudson A, Howley PM, Ploegh HL: **A human herpesvirus 7 glycoprotein, U21, diverts major histocompatibility complex class I molecules to lysosomes**. *J Virol* 2001, **75**:12347-12358 PMID: 11711625..

This paper demonstrates a function for the HHV-7 U21 gene product.

25. •• May NA, Wang Q, Balbo A, Konrad SL, Buchli R, Hildebrand WH, Schuck P, Hudson A:: **HHV-7 U21 tetramerizes to associate with class I major histocompatibility complex molecules**. *Journal of virology* 2014, **88** PMID: 24390327..

Here, the authors show high affinity binding of HHV-7 U21 tetramers for class I MHC molecules.

26. May NA, Glosson NL, Hudson AW: **HHV-7 U21 downregulates classical and non-classical class I MHC molecules from the cell surface**. *J Virol* 2010, **84**:3738-3751 PMID:.

27. Cosman D, Mullberg J, Sutherland C, Chin W, Armitage R, Fanslow W, Kubin M, Chalupny N:: **ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor**. *Immunity* 2001, **14**:123-133 PMID: 11239445.

28. Welte SA, Sinzger C, Lutz SZ, Singh-Jasuja H, Sampaio KL, Eknigk U, Rammensee H-G, Steinle A:: **Selective intracellular retention of virally induced NKG2D ligands by the human cytomegalovirus UL16 glycoprotein**. *Eur J Immunol* 2003, **33**:194-203 PMID: 12594848.

29. Cosman D, Mullberg J, Sutherland CL, Chin W, Armitage R, Fanslow W, Kubin M, Chalupny NJ: **ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor**. *Immunity* 2001, **14**:123-133 PMID: 11239445.

30. Li P, Morris DL, Willcox BE, Steinle A, Spies T, Strong RK: **Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA**. *Nat Immunol* 2001, **2**:443-451 PMID: 11323699.

31. Li P, Willie ST, Bauer S, Morris DL, Spies T, Strong RK: **Crystal structure of the MHC class I homolog MIC-A, a gammadelta T cell ligand**. *Immunity* 1999, **10**:577-584 PMID: 10367903.

32. Radaev S, Rostro B, Brooks AG, Colonna M, Sun PD: **Conformational plasticity revealed by the cocrystal structure of NKG2D and its class I MHC-like ligand ULBP3**. *Immunity* 2001, **15**:1039-1049 PMID: 11754823.

33. Dunn C, Chalupny NJ, Sutherland CL, Dosch S, Sivakumar PV, Johnson DC, Cosman D: **Human cytomegalovirus glycoprotein UL16 causes intracellular sequestration of NKG2D ligands, protecting against natural killer cell cytotoxicity**. *J Exp Med* 2003, **197**:1427-1439 PMID: 12782710.

34. Wu J, Chalupny NJ, Manley TJ, Riddell SR, Cosman D, Spies T: **Intracellular retention of the MHC class I-related chain B ligand of NKG2D by the human cytomegalovirus UL16 glycoprotein**. *J Immunol* 2003, **170**:4196-4200 PMID: 12682252.

35. Eagle RA, Traherne JA, Hair JR, Jafferji I, Trowsdale J: **ULBP6/RAET1L is an additional human NKG2D ligand**. *Eur J Immunol* 2009, **39**:3207-3216 PMID: 19658097.

36. Rölle A, Mousavi-Jazi M, Eriksson M, Odeberg J, Soderberg-Naucler C, Cosman D, Karre K, Cerboni C: **Effects of human cytomegalovirus infection on ligands for the activating NKG2D receptor of NK cells: up-regulation of UL16-binding protein (ULBP)1 and ULBP2 is counteracted by the viral UL16 protein**. *J Immunol* 2003, **171**:902-908 PMID: 12847260.

37. •• Schneider CL, Hudson A: **The human herpesvirus-7 (HHV-7) U21 immunoevasin subverts NK-mediated cytoxicity through modulation of MICA and MICB**. *PLoS Pathog* 2011, **7**:e1002362 PMID: 22102813..

This paper shows dual function of HHV-7 U21 in downregulating NK-activating ligands as well as class I MHC molecules.

38. •• Glosson NL, Hudson A: **Human herpesvirus-6A and -6B encode viral immunoevasins that downregulate class I MHC molecules**. *Virology* 2007, **365**:125-135 PMID: 17467766..
This paper demonstrates that HHV-6A U21 and HHV-6B U21 can also bind to and reroute class I MHC molecules to lysosomes, although not as efficiently as HHV-7 U21.

39. Breckenridge DG, Xue D:: **Regulation of mitochondrial membrane permeabilization by BCL-2 family proteins and caspases**. *Current opinion in cell biology* 2004, **16**:647-652 PMID: 15530776.

40. Galluzzi L, Brenner C, Morselli E, Touat Z, Kroemer G: **Viral control of mitochondrial apoptosis**. *PLoS Pathogens* 2008, **4**:e1000018 PMID: 18516228.

41. •• Kofod-Olsen E, Ross-Hansen K, Schleimann MH, Jensen DK, Møller JML, Bundgaard B, Mikkelsen JG, Höllsberg P: **U20 Is Responsible for Human Herpesvirus 6B Inhibition of TNF Receptor-Dependent Signaling and Apoptosis**. *J Virol* 2012. PMID: 22896603..
This paper shows that HHV-6B U20 expression inhibits PARP cleavage induced by extrinsic ligation of TNF receptor 1.

42. •• Kofod-Olsen E, Møller JML, Schleimann MH, Bundgaard B, Bak RO, ØSter B, Mikkelsen JG, Hupp T, Höllsberg P: **Inhibition of p53-Dependent, but Not p53-Independent, Cell Death by U19 Protein from Human Herpesvirus 6B**. *PLoS ONE* 2013, **8**:e59223.
Here, the authors demonstrate inhibition of intrinsically induced apoptosis by the HHV-6B U19 gene product.

43. Abate DA, Watanabe S, Mocarski ES: **Major human cytomegalovirus structural protein pp65 (ppUL83) prevents interferon response factor 3 activation in the interferon response**. *J Virol* 2004, **78**:10995-11006 PMID: 15452220.

44. Browne EP, Shenk T: **Human cytomegalovirus UL83-coded pp65 virion protein inhibits antiviral gene expression in infected cells**. *Proc Natl Acad Sci USA* 2003, **100**:11439-11444 PMID: 12972646.

45. Li T, Chen J, Cristea IM: **Human cytomegalovirus tegument protein pUL83 inhibits IFI16-mediated DNA sensing for immune evasion**. *Cell, Host Microbe* 2013, **14**:591-599 PMID: 24237704.

46. Paulus C, Krauss S, Nevels M: **A human cytomegalovirus antagonist of type I IFN-dependent signal transducer and activator of transcription signaling**. *Proc Natl Acad Sci USA* 2006, **103**:3840-3845 PMID:.

47. Krauss S, Kaps J, Czech N, Paulus C, Nevels M: **Physical requirements and functional consequences of complex formation between the cytomegalovirus IE1 protein and human STAT2**. *J Virol* 2009, **83**:12854-12870 PMID: 19812155.

48. Taylor RT, Bresnahan WA: **Human cytomegalovirus IE86 attenuates virus- and tumor necrosis factor alpha-induced NFkappaB-dependent gene expression**. *J Virol* 2006, **80**:10763-10771 PMID: 17041226.

49. Taylor RT, Bresnahan WA: **Human cytomegalovirus immediate-early 2 gene expression blocks virus-induced beta interferon production**. *J Virol* 2005, **79**:3873-3877 PMID: 15731283.

50. Child S, Jarrahian S, Harper V, Geballe A: **Complementation of Vaccinia Virus Lacking the Double-Stranded RNA-Binding Protein Gene E3L by Human Cytomegalovirus**. *J Virol* 2002, **76**:4912-4918 PMID:.

51. Child SJ, Hakki M, De Niro KL, Geballe AP: **Evasion of Cellular Antiviral Responses by Human Cytomegalovirus TRS1 and IRS1**. *J Virol* 2003, **78**:197-205 PMID:.

52. Hakki M, Geballe AP: **Double-Stranded RNA Binding by Human Cytomegalovirus pTRS1**. *J Virol* 2005, **79**:7311-7318 PMID:.

53. Hakki M, Marshall EE, De Niro KL, Geballe AP: **Binding and Nuclear Relocalization of Protein Kinase R by Human Cytomegalovirus TRS1**. *J Virol* 2006, **80**:11817-11826 PMID:.

54. Tan JCG, Avdic S, Cao JZ, Mocarski ES, White KL, Abendroth A, Slobedman B: **Inhibition of 2'5'-oligoadenylate synthetase expression and function by the human cytomegalovirus ORF94 gene product**. *J Virol* 2011, **85**:5696-5700 PMID: 21450824.

55. Poole E, Atkins E, Nakayama T, Yoshie O, Groves I, Alcamí A, Sinclair J: **NF- B-Mediated Activation of the Chemokine CCL22 by the Product of the Human Cytomegalovirus Gene UL144 Escapes Regulation by Viral IE86**. *J Virol* 2008, **82**:4250-4256 PMID:.

56. Poole E, King CA, Sinclair JH, Alcami A: **The UL144 gene product of human cytomegalovirus activates NFkappaB via a TRAF6-dependent mechanism**. *EMBO J* 2006, **25**:4390-4399 PMID: 16932746.

57. Cheung TC, Humphreys IR, Potter KG, Norris PS, Shumway HM, Tran BR, Patterson G, Jean-Jacques R, Yoon M, Spear PG, Murphy KM, Lurain NS, Benedict CA, Ware CF: **Evolutionarily divergent herpesviruses modulate T cell activation by targeting the herpesvirus entry mediator cosignaling pathway**. *Proc Natl Acad Sci USA* 2005, **102**:13218-13223 PMID: 16131544.

58. Kotenko SV, Saccani S, Izotova LS, Mirochnitchenko OV, Pestka S: **Human cytomegalovirus harbors its own unique IL-10 homolog (cmvIL-10)**. *Proc Natl Acad Sci USA* 2000, **97**:1695-1700 PMID: 10677520.

59. Nachtwey J, Spencer JV: **HCMV IL-10 suppresses cytokine expression in monocytes through inhibition of nuclear factor-kappaB**. *Viral Immunol* 2008, **21**:477-482 PMID: 19115937.

60. Wang D, Bresnahan W, Shenk T: **Human cytomegalovirus encodes a highly specific RANTES decoy receptor**. *Proc Natl Acad S ci USA* 2004, **101**:16642-16647 PMID:.

61. Bodaghi B, Jones TR, Zipeto D, Vita C, Sun L, Laurent L, Arenzana-Seisdedos F, Virelizier JL, Michelson S: **Chemokine sequestration by viral chemoreceptors as a novel viral escape strategy: withdrawal of chemokines from the environment of cytomegalovirus-infected cells**. *J Expl Med* 1998, **188**:855-866 PMID: 9730887.

62. Tschische P, Tadagaki K, Kamal M, Jockers R, Waldhoer M: **Heteromerization of human cytomegalovirus encoded chemokine receptors**. *Biochem Pharm* 2011, **82**:610-619 PMID: 21684267.

63. Billstrom MA, Johnson GL, Avdi NJ, Worthen GS: **Intracellular signaling by the chemokine receptor US28 during human cytomegalovirus infection**. *J Virol* 1998, **72**:5535-5544 PMID: 9621010.

64. Billstrom MA, Lehman LA, Scott Worthen G: **Depletion of extracellular RANTES during human cytomegalovirus infection of endothelial cells**. *Am J Resp Cell Mol Biol* 1999, **21**:163-167 PMID: 10423397.

65. Randolph-Habecker J-R, Rahill B, Torok-Storb B, Vieira J, Kolattukudy PE, Rovin BH, Sedmak DD: **The expression of the cytomegalovirus chemokine receptor homolog US28 sequesters biologically active CC chemokines and alters IL-8 production**. *Cytokine* 2002, **19**:37-46 PMID: 12200112.

66. Waldhoer M, Kledal TN, Farrell H, Schwartz TW: **Murine cytomegalovirus (CMV) M33 and human CMV US28 receptors exhibit similar constitutive signaling activities**. *J Virol* 2002, **76**:8161-8168 PMID: 12134021.

67. Tadagaki K, Tudor D, Gbahou F, Tschische P, Waldhoer M, Bomsel M, Jockers R, Kamal M: **Human cytomegalovirus-encoded UL33 and UL78 heteromerize with host CCR5 and CXCR4 impairing their HIV coreceptor activity**. *Blood* 2012, **119**:4908-4918 PMID: 22496149.

68. Lüttichau HR: **The Cytomegalovirus UL146 Gene Product vCXCL1 Targets Both CXCR1 and CXCR2 as an Agonist**. *J Biol Chem* 2010, **285**:9137-9146 PMID:.

69. Penfold ME, Dairaghi DJ, Duke GM, Saederup N, Mocarski ES, Kemble GW, Schall TJ: **Cytomegalovirus encodes a potent alpha chemokine**. *Proc Natl Acad Sci USA* 1999, **96**:9839-9844 PMID: 10449781.

70. Akter P, Cunningham C, McSharry BP, Dolan A, Addison C, Dargan DJ, Hassan-Walker AF, Emery VC, Griffiths PD, Wilkinson GWG, Davison AJ: **Two novel spliced genes in human cytomegalovirus**. *J Gen Virol* 2003, **84**:1117-1122 PMID: 12692276.

71. Hui-hui G, Ran T, Qi Z, Jun X, Shi-qiang S: **Recombinant HCMV UL128 expression and functional identification of PBMC-attracting activity in vitro**. *Arch Virol* 2012, **158**:173-177 PMID:.

72. Wiertz EJ, Tortorella D, Bogyo M, Yu J, Mothes W, Jones TR, Rapoport TA, Ploegh HL: **Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction**. *Nature* 1996, **384**:432-438 PMID:.

73. Wiertz EJ, Jones TR, Sun L, Bogyo M, Geuze HJ, Ploegh HL: **The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol**. *Cell* 1996, **84**:769-779 PMID: 8625414.

74. Ahn K, Angulo A, Ghazal P, Peterson PA, Yang Y, Fruh K: **Human cytomegalovirus inhibits antigen presentation by a sequential multistep process**. *Proc Natl Acad Sci USA* 1996, **93**:10990-10995 PMID:.

75. Jones TR, Wiertz EJ, Sun L, Fish KN, Nelson JA, Ploegh HL: **Human cytomegalovirus US3 impairs transport and maturation of major histocompatibility complex class I heavy chains**. *Proc Natl Acad Sci USA* 1996, **93**:11327-11333 PMID:.

76. Cox JH, Bennink JR, Yewdell JW: **Retention of adenovirus E19 glycoprotein in the endoplasmic reticulum is essential to its ability to block antigen presentation**. *J Exp Med* 1991, **174**:1629-1637 PMID:.

77. Ziegler H, Thale R, Lucin P, Muranyi W, Flohr T, Hengel H, Farrell H, Rawlinson W, Koszinowski UH: **A mouse cytomegalovirus glycoprotein retains MHC class I complexes in the ERGIC/cis-Golgi compartments**. *Immunity* 1997, **6**:57-66 PMID:.

78. Fruh K, Ahn K, Djaballah H, Sempe P, van Endert PM, Tampe R, Peterson PA, Yang Y: **A viral inhibitor of peptide transporters for antigen presentation**. *Nature* 1995, **375**:415-418 PMID: 7760936.

79. Hill A, Jugovic P, York I, Russ G, Bennink J, Yewdell J, Ploegh H, Johnson D: **Herpes simplex virus turns off the TAP to evade host immunity**. *Nature* 1995, **375**:411-415 PMID:.

80. Fielding CA, Aicheler R, Stanton RJ, Wang ECY, Han S, Seirafian S, Davies J, McSharry BP, Weekes MP, Antrobus PR, Prod'homme V, Blanchet FP, Sugrue D, Cuff S, Roberts D, Davison AJ, Lehner PJ, Wilkinson GWG, Tomasec P: **Two Novel Human Cytomegalovirus NK Cell Evasion Functions Target MICA for Lysosomal Degradation**. *PLoS Pathogens* 2014, **10**:e1004058 PMID: 24787765.

81. Ashiru O, Bennett NJ, Boyle LH, Thomas M, Trowsdale J, Wills MR: **NKG2D ligand MICA is retained in the cis-Golgi apparatus by human cytomegalovirus protein UL142**. *J Virol* 2009, **83**:12345-12354 PMID: 19793804.

82. Chalupny NJ, Rein-Weston A, Dosch S, Cosman D: **Down-regulation of the NKG2D ligand MICA by the human cytomegalovirus glycoprotein UL142**. *Biochem Biophys Res Comm* 2006, **346**:175-181 PMID:.

83. Prod'homme V, Sugrue DM, Stanton RJ, Nomoto A, Davies J, Rickards CR, Cochrane D, Moore M, Wilkinson GWG, Tomasec P: **Human cytomegalovirus UL141 promotes efficient downregulation of the natural killer cell activating ligand CD112**. *J Gen Virol* 2010, **91**:2034-2039 PMID: 20410314.

84. Tomasec P, Wang ECY, Davison AJ, Vojtesek B, Armstrong M, Griffin C, McSharry BP, Morris RJ, Llewellyn-Lacey S, Rickards C, Nomoto A, Sinzger C, Wilkinson GWG: **Downregulation of natural killer cell-activating ligand CD155 by human cytomegalovirus UL141**. *Nature Immunol* 2005, **6**:181-188 PMID: 15640804.

85. Stern-Ginossar N, Elefant N, Zimmermann A, Wolf DG, Saleh N, Biton M, Horwitz E, Prokocimer Z, Prichard M, Hahn G, Goldman-Wohl D, Greenfield C, Yagel S, Hengel H, Altuvia Y, Margalit H, Mandelboim O: **Host immune system gene targeting by a viral miRNA**. *Science* 2007, **317**:376-381 PMID: 17641203.

86. Fahnestock ML, Johnson JL, Feldman RM, Neveu JM, Lane WS, Bjorkman PJ: **The MHC class I homolog encoded by human cytomegalovirus binds endogenous peptides**. *Immunity* 1995, **3**:583-590 PMID: 7584148.

87. Reyburn HT, Mandelboim O, Vales-Gomez M, Davis DM, Pazmany L, Strominger JL: **The class I MHC homologue of human cytomegalovirus inhibits attack by natural killer cells**. *Nature* 1997, **386**:514-517 PMID:.

88. Arnon TI, Achdout H, Levi O, Markel G, Saleh N, Katz G, Gazit R, Gonen-Gross T, Hanna J, Nahari E, Porgador A, Honigman A, Plachter B, Mevorach D, Wolf DG, Mandelboim O: **Inhibition of the NKp30 activating receptor by pp65 of human cytomegalovirus**. *Nature Immunol* 2005, **6**:515-523 PMID:.

89. Yu Y, Alwine JC: **Human cytomegalovirus major immediate-early proteins and simian virus 40 large T antigen can inhibit apoptosis through activation of the phosphatidylinositide 3'-OH kinase pathway and the cellular kinase Akt**. *J Virol* 2002, **76**:3731-3738 PMID: 11907212.

90. Taylor RT, Bresnahan WA: **Human cytomegalovirus immediate-early 2 protein IE86 blocks virus-induced chemokine expression**. *J Virol* 2006, **80**:920-928 PMID: 16378994.

91. Smith W, Tomasec P, Aicheler R, Loewendorf A, Nemčovičová I, Wang ECY, Stanton RJ, Macauley M, Norris P, Willen L, Ruckova E, Nomoto A, Schneider P, Hahn G, Zajonc DM, Ware CF, Wilkinson GWG, Benedict CA: **Human cytomegalovirus glycoprotein UL141 targets the TRAIL death receptors to thwart host innate antiviral defenses**. *Cell Host Microbe* 2013, **13**:324-335 PMID: 23498957.

92. Skaletskaya A, Bartle LM, Chittenden T, McCormick AL, Mocarski ES, Goldmacher VS: **A cytomegalovirus-encoded inhibitor of apoptosis that suppresses caspase-8 activation**. *Proc Natl Acad Sci USA* 2001, **98**:7829-7834 PMID: 11427719.

93. McCormick AL, Roback L, Livingston-Rosanoff D, St Clair C: **The human cytomegalovirus UL36 gene controls caspase-dependent and -independent cell death programs activated by infection of monocytes differentiating to macrophages**. *J Virol* 2010, **84**:5108-5123 PMID: 20219915.

94. Goldmacher VS, Bartle LM, Skaletskaya A, Dionne CA, Kedersha NL, Vater CA, Han Jw, Lutz RJ, Watanabe S, Cahir McFarland ED, Kieff ED, Mocarski ES, Chittenden T: **A cytomegalovirus-encoded mitochondria-localized inhibitor of apoptosis structurally unrelated to Bcl-2**. *Proc Natl Acad Sci USA* 1999, **96**:12536-12541 PMID: 10535957.

95. Zhang A, Hildreth RL, Colberg-Poley AM: **Human cytomegalovirus inhibits apoptosis by proteasome-mediated degradation of Bax at endoplasmic reticulum-mitochondrion contacts**. *J Virol* 2013, **87**:5657-5668 PMID: 23487455.

96. Terhune S, Torigoi E, Moorman N, Silva M, Qian Z, Shenk T, Yu D: **Human cytomegalovirus UL38 protein blocks apoptosis**. *J Virol* 2007, **81**:3109-3123 PMID: 17202209.

97. Qian Z, Xuan B, Gualberto N, Yu D: **The human cytomegalovirus protein pUL38 suppresses endoplasmic reticulum stress-mediated cell death independently of its ability to induce mTORC1 activation**. *J Virol* 2011, **85**:9103-9113 PMID: 21715486.

98. Pleskoff O, Casarosa P, Verneuil L, Ainoun F, Beisser P, Smit M, Leurs R, Schneider P, Michelson S, Ameisen JC: **The human cytomegalovirus-encoded chemokine receptor US28 induces caspase-dependent apoptosis**. *FEBS Journal* 2005, **272**:4163-4177 PMID:.

99. Babu SG, Pandeya A, Verma N, Shukla N, Kumar RV, Saxena S: **Role of HCMV miR-UL70-3p and miR-UL148D in overcoming the cellular apoptosis**. *Mol Cell Biochem* 2014, **393**:89-98 PMID: 24737391.

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Current Opinion in Virology

Roseoloviruses: unmet needs and research priorities Perspective

Mary T Caserta[1], Laurie T Krug[2] and Philip E Pellett[3]

The human roseoloviruses, human herpesviruses 6A (HHV-6A), HHV-6B, and HHV-7, are highly prevalent viruses that typically cause fever/rash illnesses such as roseola during early life primary infections. They also cause significant neurologic disease and complications following stem cell and solid organ transplantation, and have suggestive but less certain etiologic associations with other neurologic diseases and immunologic disorders. The US National Institute of Allergy and Infectious Diseases recently sponsored a workshop (Roseoloviruses: Clinical Impact, Interventions, and Research Needs) to discuss disease associations, novel biology, and the many unmet research needs related to Roseoloviruses. This perspective is a distillation of the workshop's presentations and discussions, with a focus on the more general research priorities that emerged.

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Edited by **Laurie Krug**

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The National Institute of Allergy and Infectious Diseases of the US National Institutes of Health recently sponsored a workshop entitled 'Roseoloviruses: Clinical Impact, Interventions, and Research Needs.' The meeting brought together over 50 clinical, translational, and basic science researchers, as well as scientific program officials to discuss the growing list of robust disease associations, novel biology, and unmet research needs related to Roseoloviruses.

The human Roseoloviruses are human herpesviruses (HHV) 6A, HHV-6B, and HHV-7 [1••]. These viruses are distinct, yet closely related members of the Roseolovirus genus of the Betaherpesvirus subfamily. They are ubiquitous, infecting upwards of 95% of the human population; HHV-6B is best known as the major etiologic agent of roseola infantum and febrile illnesses in early childhood.

Roseoloviruses are also associated with more consequential pathologies, including neurological diseases and complications of both hematopoietic and solid organ transplantation. Although HHV-6 has long been listed as an Emerging Infectious Pathogen by the NIH, there remains a paucity of information about roseolovirus infections, and the full spectrum of diseases caused by roseoloviruses is not known. Limited understanding of their pathogenesis has slowed development of antiviral agents and therapeutic strategies. Unique features of roseolovirus biology, such as germline chromosomal integration of HHV-6 (ciHHV-6) in 1% of the human population have complicated the development of diagnostics and raise important biological questions.

To address these gaps, the workshop was convened to summarize the known health and societal impacts of roseolovirus infections, to identify critical barriers to the development of novel antiviral therapeutics and diagnostics, and to define high priority research questions. Articles by the meeting's speakers and their colleagues in this special issue of *Current Opinions in Virology* provide in depth summaries of the state of the art, and elaborate on research priorities for topics covered at the meeting (Table 1). This perspective is a distillation of the workshop's presentations and lively discussions, with a focus on the more general research priorities that emerged (Table 2).

Expanding recognition of the significant clinical impacts of roseolovirus infections

Primary HHV-6B and HHV-7 infections cause *roseola infantum*, or sixth disease. In addition, the roseoloviruses are neuropathogenic in immunocompetent and immunocompromised hosts. Reactivation of HHV-6B is a frequent complication of hematopoietic stem cell (HCT) transplantation, and the syndrome of post-transplant acute limbic encephalitis (PALE) is a recognized complication of HHV-6B reactivation in the early post-transplant period. This, plus the finding that 30% of febrile status epilepticus events in children are concurrent with periods of active infection with HHV-6B, adds to the previously noted connection between primary HHV-6B infection in children and febrile seizures.

Other diseases, including encephalitis in immunocompetent hosts, acute graft versus host disease, fever and rash

Table 1

Roseolovirus workshop: speakers and topics[a].

Roseoloviruses: time for action (Robert C. Gallo, Institute for Human Virology)
Roseolovirus impacts and needs: a community perspective (Kristin Loomis, Human Herpesvirus 6 Foundation)
Clinical impact of primary infections with roseoloviruses (Mary Caserta, University of Rochester) [2]
Roseoloviruses in immune compromised patients: impacts and prospects for clinical trials (Danielle Zerr, University of Washington) [3]
Neurologic complications of roseoloviruses in immune competent children (Leon Epstein, Northwestern University) [2]
The path to therapeutics for roseolovirus infections (Richard J. Whitley, University of Alabama at Birmingham) [4]
Diagnosis of roseolovirus infections: what we know we don't know (Keith Jerome, University of Washington) [5]
Roseoloviruses: a treasure trove of immune modulators (Amy Hudson, Medical College of Wisconsin) [6]
Chromosomally integrated HHV-6: impacts on virus, cell, and organismal biology (Louis Flamand, Université Laval) [7]
Animal Models (Paolo Lusso, National Institute of Allergy and Infectious Diseases) [8]
Roseomics: a blank slate (Eain Murphy, Cleveland Clinic) [9]

[a] References are provided for review articles prepared by the speakers and their colleagues for inclusion in this issue of *Current Opinions in Virology*. The special issue contains additional related articles [10–12].

following HCT, myelosuppression following HCT, hepatitis, allograft dysfunction, and pneumonitis have been associated with HHV-6, but these links remain tenuous. Their confirmation has been complicated by the ubiquitous nature of Roseolovirus infections, which are characterized by lifelong latency or persistence at several body sites. A further complication is that approximately 1% of the population harbors ciHHV-6.

An important research priority highlighted at the meeting is the development of rigorous criteria for identifying end-organ disease caused by primary or reactivated roseolovirus infection. Detection of viral DNA in blood or body fluids is informative, but moving beyond simple associations to proof of causality will require much more. Two approaches were suggested: (1) use of a specific treatment for HHV-6B disease (e.g., an antiviral or immunotherapeutic) to link viral replication with pathology, and (2) in-depth molecular interrogation of tissues involved in end-organ disease to identify pathologic and molecular signatures of HHV-6 infection.

Given the recent recognition of ciHHV-6, case reports associating high loads of HHV-6A or HHV-6B with disease should be reconsidered. Studies are needed to elucidate the mechanisms and pathogenic effects of ciHHV-6 in normal and immunocompromised hosts. Such information will impact not only individuals with ciHHV-6, but is also needed to assess the effects of ciHHV-6 on organ transplants.

Need for improved diagnostics and therapeutics

Molecular methods, including digital PCR, can identify ciHHV-6 and distinguish it from other forms of HHV-6 infection. Strides made in understanding the pathogenesis of cytomegalovirus disease illustrate how standardized units of DNA detection would improve our understanding of roseolovirus pathogenesis. Standardized measurement of viral DNA levels are needed to define clinically-relevant viral loads and for meaningful cross-institutional comparisons of viral loads. Further development and standardization of DNA qPCR and reverse

Table 2

Major basic science needs and clinical research objectives.

Foundational research objectives
Assign functions to unknown genes and examine molecular interactions with host cells.
Standardize clinical isolates and cell culture infection systems.
Generate roseolovirus bacterial artificial chromosomes for genetic manipulation of virus.
Develop a broad panel of monoclonal antibodies.
Apply modern 'omics' methods to understand roseolovirus genomic variation, gene expression, and effects on host cell gene expression and activity.
Define the molecular mechanisms and consequences of Roseolovirus chromosomal integration.
Develop animal models to understand roseolovirus pathogenesis and evaluate therapeutic approaches.
Clinical research objectives
Epidemiologic studies to understand the natural history and clinical significance of ciHHV-6.
Rigorously evaluate associations between end organ disease and roseolovirus infection.
Standardize reliable DNA qPCR and RNA RT-qPCR assays with clinically relevant thresholds.
Interrogate cells and tissues for active viral infection or biomarkers of host responses to viral infection.
Robust clinical trials or longitudinal studies to prove or disprove disease associations.
Must distinguish between HHV-6A and HHV-6B.
Must define latent versus active infection and account for ciHHV-6.
Antiviral drug or immune therapy should result in reduced viral burden.

transcriptase-quantitative PCR assays are needed to enable discrimination between active and latent infections. New diagnostic tools are also needed for rapid detection and diagnosis of primary infection. Technologies suitable for point-of-care testing have the potential to alleviate unnecessary testing and antibiotic misuse. Such tools are critical precursors for the development and evaluation of scientifically based management and treatment protocols.

Only small-scale prophylaxis studies in HCT recipients have been published to date, and no antiviral drug is specifically licensed to treat HHV-6B disease. Newer compounds undergoing evaluation for the treatment of cytomegalovirus infections might prove useful for treatment of roseolovirus infections. A controlled therapeutic trial is needed for PALE and to determine whether prevention or inhibition of HHV-6B reactivation improves outcomes following transplantation. Unfortunately, a vicious cycle is at play, as clinical trials are needed to prove causality between infection and disease, while at the same time well-established pathologic associations are needed to justify drug development and clinical trials. Breaking this 'log jam' was recognized as one of the greatest challenges to the field.

Major gaps in understanding roseolovirus molecular virology and pathogenesis

State-of-the-art technologies must be applied to better define the host–pathogen 'interactome.' In-depth genomic analyses of uncultured clinical strains of roseoloviruses will help to identify possible disease-specific pathogenesis determinants. A multi-center repository of viral and human specimens from longitudinal studies is needed to enable such studies. Proteomics and metabolomics can open the door to identification of unique viral functions that would inform pathogenesis and serve as targets for new therapeutics. A basic molecular infrastructure of genetic systems, libraries of recombinant viruses, cloned ORFs for screening, and monoclonal antibodies is needed to define mechanisms by which roseoloviruses modulate host biology (e.g., immune responses) at the cellular and organismal levels. Animal models that recapitulate human disease are being developed in mice and non-human primates.

Time to act

Rigorous proof of etiologic associations of these ubiquitous but neglected human pathogens will require coordinated investment in improved diagnostic methods, and development of safe and specific antivirals. Therapeutic approaches will need to be evaluated in well-designed, controlled trials that include meaningful clinical and virologic endpoints. In addition, and as has been the case for other important pathogens, a solid understanding of basic roseolovirus biology is an essential critical underpinning for future work.

A roadmap has been drawn. It is time to move forward.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- •• of outstanding interest

1. •• Yamanishi K, Mori Y, Pellett PE: **Human herpesviruses 6 and 7**. In *Fields Virology*, edn 6. Edited by Knipe DM, Howley PM, Cohen JI, Griffin DE, Lamb RA, Martin MA, Racaniello VR, Roizman B.Philadelphia: Lippincott Williams & Wilkins; 2013:2058-2079.
This chapter provides broad background and many historical references.

2. Tesini B, Epstein LG, Caserta MT: **Clinical impact of primary infection with Roseoloviruses**. *Curr Opin Virol* 2014, **9C**.

3. Hill JA, Zerr DM: **Roseoloviruses in transplant recipients: clinical consequences and prospects for treatment and prevention trials**. *Curr Opin Virol* 2014, **9C**:53-60.

4. Prichard MN, Whitley RJ: **Development of new therapies for human herpesvirus 6**. *Curr Opin Virol* 2014, **9C**.

5. Hill JA, Sedlak RH, Jerome K: **Past, present, and future perspectives on the diagnosis of Roseolovirus infections**. *Curr Opin Virol* 2014, **9C**.

6. Hudson AP: **Roseoloviruses: a treasure trove of immune modulators**. *Curr Opin Virol* 2014, **9C**.

7. Kaufer BB, Flamand L: **Chromosomally integrated HHV-6: impacts on virus, cell, and organismal biology**. *Curr Opin Virol* 2014, **9C**.

8. Horvat B, Berges BK, Lusso P: **Recent developments in animal models for human herpesvirus 6A and 6B**. *Curr Opin Virol* 2014, **9C**.

9. Moorman NJ, Murphy E: **Roseomics: a blank slate**. *Curr Opin Virol* 2014, **9C**.

10. Shamir E, Zeigerman H, Frenkel N: **Roseoloviruses: viral host interactions and cell cycle arrest**. *Curr Opin Virol* 2014, **9C**.

11. Leibovitch E, Jacobson S: **An update on the evidence linking HHV-6 with multiple sclerosis**. *Curr Opin Virol* 2014:9C.

12. Becerra A, Gibson L, Stern LJ, Calvo-Calle JM: **Immune response to HHV-6 and implications for immunotherapy**. *Curr Opin Virol* 2014, **9C**.

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Current Opinion in Virology

Roseoloviruses manipulate host cell cycle

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During lytic infections HHV-6A and HHV-6B disrupt E2F1–Rb complexes by Rb degradation, releasing E2F1 and driving the infected cells toward the S-phase. Whereas upon infection E2F1 and its cofactor DP1 were up-regulated, additional E2F responsive genes were expressed differentially in various cells. E2F binding sites were identified in promoters of several HHV-6 genes, including the U27 and U79 associated with viral DNA replication, revealing high dependence on the binding site and the effect of the E2F1 transcription factor. Viral genes regulation by E2F1 can synchronize viral replication with the optimal cell cycle phase, enabling utilization of host resources for successful viral replication. Furthermore, it was found that infection by roseoloviruses leads to cell cycle arrest, mostly in the G2/M-phase.

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Introduction

The mammalian cell cycle is a highly regulated process. At the G1 phase cells undergo a critical check point, ensuring their readiness for DNA synthesis. This is followed by the S-phase during which the cellular genome is duplicated. Following DNA duplication, the cell progresses into the G2 phase, preparing for mitosis. Members of the E2F family serve as transcriptional activators of genes that play significant roles in cell cycle control, including: DNA replication, mitosis, the mitotic checkpoint, DNA-damage checkpoints, DNA repair, differentiation, development and apoptosis [1]. In responsive genes containing the E2F binding sequence transcription begins by binding the E2F and DP heterodimers to the E2F binding site. The E2F-DP transcription complexes are negatively regulated by members of the retinoblastoma (Rb) protein family, which block the E2F activation domain, preventing the transcription of E2F responsive genes.

The Rb protein is regulated by phosphorylation and degradation. Hypophosphorylated Rb binds E2F1 with a high affinity, leading to inhibition of E2F1 transcription activity. In the G1 phase the Rb protein is inactivated following its phosphorylation by cyclin D/CDK-4/6 and cyclin E/CDK-2 complexes, resulting in its dissociation from E2F1 and cellular entry into the S-phase [2,3].

DNA viruses synthesize significant quantities of nucleic acid during the productive lytic replication. Therefore they have evolved ways to modulate the Rb–E2F pathway. Viral inactivation of the Rb family members enables them to create an environment more accommodating for viral replication. The disassembly of Rb/E2F1 complexes by viral proteins leads to accumulation of free E2F1 transcription factor and induction of S phase entrance. Such viral proteins include the extensively studied human papillomavirus oncoprotein E7, the adenovirus E1A protein, and the SV40 large T antigen. These three viral proteins represent two distinct mechanisms of Rb inactivation: steric disruption of Rb–E2F complexes and Rb degradation [4••].

Herpesviruses encode proteins that use these pathways and additional direct and indirect mechanisms to inhibit Rb family member function.

Cells infected by the ***alphaherpesviruses*** HSV-1, HSV-2, and VZV, accumulate in the G1 phase of the cell cycle [5,6]. Moreover, while the Rb proteins remain unphosphorylated in HSV-infected cells, the activity of kinases responsible for their phosphorylation, the Cdks, is critical for HSV-1 replication [7]. Thus, the *alphaherpesviruses* may be less dependent on cellular E2F-responsive genes for viral DNA replication than other herpesviruses and may not need to target Rb family members for inactivation.

The ***gammaherpesviruses*** are associated with proliferative disorders including a number of cancers. Both EBV and KSHV appear to encode proteins that modulate the Rb–E2F pathway, either directly or indirectly. EBV has multiple proteins (Z, R, LMP-1, EBNA-2,-3C,-5), that could lead to the phosphorylation of Rb by cellular CDKs, and/or may directly phosphorylate Rb through the function of the viral kinase, BGLF-4 (ortholog of HCMV UL97). Moreover, EBV at the lytic infection, but not during latency, was shown to induce cell cycle arrest at

the G1/S with elevated levels of cyclin E and cyclin A [8]. In the KSHV infection E2F1 pathway is activated by LANA-mediated disruption of E2F1/Rb complexes, or by direct phosphorylation of Rb through the action of the v-cyclin and/or the ORF36 proteins [9,10].

Beta-herpesviruses manipulate E2F–Rb pathway during lytic infection

For the HCMV: It was demonstrated that at very early stages of infection hypophosphorylated Rb protein was first degraded, and protein synthesized de novo was then hyperphosphorylated [11]. The HCMV pp71 protein is a prominent component of the viral tegument [12,13]. By binding to Rb protein, pp71 induces Rb degradation in a proteasome-dependent, ubiquitin-independent manner [14]. HCMV UL97 has been shown to phosphorylate multiple residues of Rb, disrupting the E2F1/Rb and Rb/HDAC complexes, rendering Rb inactive. Due to both pp71-mediated degradation and UL97-mediated phosphorylation, Rb is inactivated and E2F responsive genes are highly expressed [15].

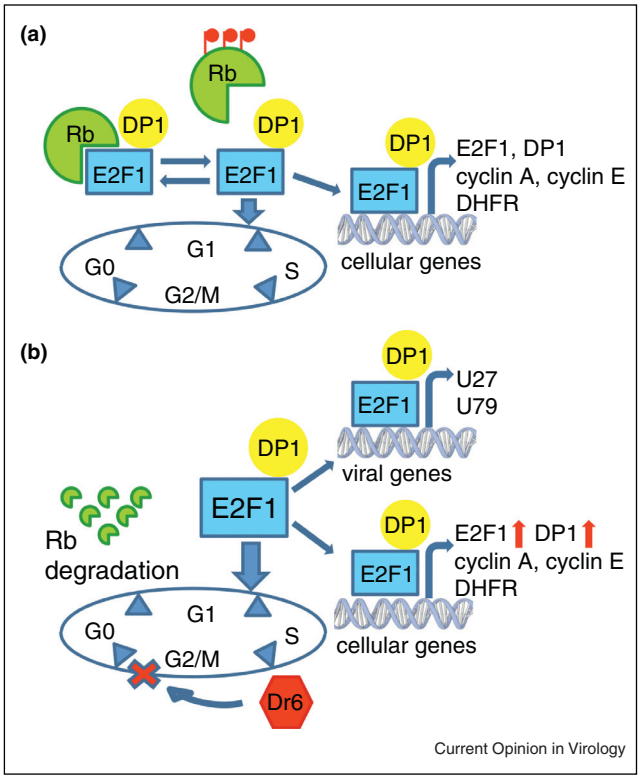
For the Roseoloviruses: We have shown [16•,17••] that in SupT1 T cells infected with HHV-6A the E2F1 protein and its co-factor DP1 increased whereas the Rb protein underwent massive degradation without hyperphosphorylation at 3 sites, Ser-780, Ser-807 and Thr-821, known to control E2F/Rb association (Figure 1). The degradation of Rb started simultaneously with E2F1 and DP1 up-regulation. Furthermore, it correlated with the accumulation of the viral p41 protein that functions in viral DNA replication. Increased E2F1 expression was also described employing a microarray assay of HHV-6B infected adult T-cell leukemia cell line [18]. Because HHV-6A infection induced elevation of free E2F1, it was of interest to monitor the expression of the E2F1 target genes and alterations of cell cycle during the infection. Although E2F1 and DP1 levels were elevated we found that cyclin A, cyclin E, DHFR and MCM5 were not up-regulated [16•,17••]. These results differ from the results of De Bolle and coworkers, who found that late post HHV-6A infection of human cord blood mononuclear cells there was increased accumulation of cyclin A without up-regulation of cyclin E [19]. Furthermore, analysis of the regulatory proteins which are involved in the cell cycle in HSB-2 cells [20], indicated that cyclins A2, B1, E1 and MCM5 were increased in HHV-6-infected cells, but there was no difference in cyclin D1. Hence, the expression of E2F1 target genes during HHV-6A infection varies in different cells/tissues examined.

Enhanced transcription of viral genes by E2F1

As described above, HHV-6A infection induces Rb degradation, up-regulation of E2F1 and DP1. However, there was no increased expression of additional E2F1-responsive genes. It was thus of interest to test whether the virus exploited E2F1 for viral gene transcription. Scanning of the HHV-6A genome revealed several genes that contained the consensus E2F binding sequence TTTSSCGC, where S is either a G or a C upstream of the ATG start codon. This included: (i) U18, a transcriptional regulator in the IE-B/E gene class. (ii) U33, a viral tegument protein that is a critical mediator of metabolic stress. (iii) U52 gene which promotes the accumulation of late transcripts. (iv) U74 gene encoding a portion of the helicase/primase complex. (v) The U27 and the U79 genes, both functioning in viral DNA synthesis [17••]. The U27 gene encodes the P41 viral DNA polymerase processivity factor [21]. The U79-80 early gene encodes a family of nuclear proteins that were found to be essential for viral DNA replication [22].

We concentrated on the U27 and U79 genes and tested whether the E2F1 transcription factor and E2F binding site were utilized for their expression [17••]. We constructed vectors containing a GFP reporter gene driven by wild-type viral promoter or by mutant promoter that

Figure 1



Mechanisms used by HHV-6 to modulate the Rb–E2F pathway and cell cycle progression. **(a)** E2F1 regulation in uninfected cells involves Rb phosphorylation and E2F1 release, leading to transcription of cellular genes and entry to S-phase. **(b)** HHV-6 infection leads to massive Rb degradation and to E2F1 release. Active E2F1 induces up-regulation of itself and its co-factor DP1 which are utilized for transcription of cellular genes as well as viral genes (U27, U79). DR6 gene product causes cell cycle arrest in G2-phase.

abolished the binding of E2Fs. It was found that the expression of the U27 promoter was dependent on the presence of the intact E2F binding site. Abolishing the E2F site led to significant decrease in promoter activity. Moreover, treatment of the cells with siRNA to E2F1 resulted in decreased U27 promoter activity whereas over-expression of E2F1 led to a substantial increase of the promoter activity. This indicated that additional E2F transcription factors may play a role in the induction of the promoter. High activity of the U79 promoter was detected in SupT1 cells depending on the presence of E2F binding site because the mutation of the site led to 90% reduced activity. The involvement of E2F1 in U79 promoter activity was demonstrated by siRNA treatment and over-expression of the E2F1 protein.

The involvement of E2F1 in transcription of the viral U27 and the U79, β and α genes, respectively, is a novel outlook of the regulation of HHV-6 gene expression. E2F1 studies are of additional interest because approximately 30% of cellular genes contain promoters with E2F1 binding site and analyses of thousand promoters revealed that more than 20% of promoters were bound by E2F1. These results place the E2F1 as a factor that contributes to the regulation of a large fraction of human genes [23]. A possible mechanism for the utilization of E2F1 transcription factor in viral replication might involve viral protein(s) recruiting the E2F1 and directing it to viral promoter(s) or to selected cellular promoters. Examples for such processes include the E2 adenovirus promoter that is activated by an E2F1/E4 complex [24]. In addition, bovine herpes virus 1 (BHV-1) infection leads to increased E2F1 protein levels and the activity of the bovine ICP0 early promoter is increased dramatically by E2F1 [25].

Cell cycle arrest in roseoloviruses infection

A variety of viruses were found to induce the G2/M arrest, including DNA viruses, RNA viruses, and retroviruses [26]. Inhibition of the G_2/M checkpoint by viruses may serve to maintain the cell in a pseudo S-like phase increasing viral replication [20]. Viruses may also utilize the DNA damage responses to maximize viral replication [27]. For the many of viruses it is difficult to define precisely the mechanism(s) of the G2/M arrest. The cell cycle is controlled by complex interactions that are not yet fully understood. In a number of instances, G2 arrest has been linked to the inhibition or delay in the activation of the Cdk1/cyclin B1 kinase activity [28]. Another mechanism is the interference with mitotic progression. It was shown that HHV-6A infection induced cell cycle arrests at the G2/M phase in different cell types, as summarized in Table 1, including: glial cell, epithelial cells, HSB-2 cells, cord blood mononuclear cells and SupT1 T cells.

HHV-6B infection led to cell cycle arrest in G1/S and/or G2/M depending on the cells that were tested [19,29–31,32•]. A new report showed that DR6 protein can induce accumulation of cells in G2/M and also the cytoplasmic accumulation of cyclin B1 [32•]. This function was dependent on the N-terminal part of the protein, which is also required for nuclear localization. Thus, a possible role of DR6 during HHV-6B infection might be that DR6 functions as a chaperone facilitating the assembly of viral replication units or facilitating cell proliferation arrest in order to enhance viral replication.

It was reported by Zauli G. and co-worker [33] that HHV-7 infection of both primary CD4+ T lymphocytes and SupT1 T-cell line induced various alteration of cell cycle regulation. Specifically, elevated level of cyclin B and Cdk1 were observed late post infection and were accompanied by G2/M arrest. To the best of our knowledge, no additional studies were reported for HHV-7 involvement in cell cycle arrest.

Mechanisms for cell cycle arrest at G2/M may involve the cellular response to DNA damage [26]. More specifically,

Table 1

Cell cycle arrest induced by HHV-6A and HHV-6B

Virus	Host cell line	Phase of arrest	Assay	Mechanism, viral protein involved	Ref.
HHV-6B	Human epithelial cell (HCT 116)	G1/S 24 hpi	FACS: tDNA, V	p53 independent	[31]
HHV-6B (DR6 exp. v.)	Human epithelial cell (HCT 116)	G2/M 24, 48 hpi	FACS: tDNA	DR6, p53 independent WB of cyclins and CDK	[32•]
HHV-6A HHV-6B	Human glial precursor cell	G1/S 72 hpi	FACS: tDNA, V	Not analyzed	[29]
HHV-6A HHV-6B	Human CBMC	G2/M 48, 72, 96 hpi	FACS: tDNA, V	WB of cyclins and CDKs	[19]
HHV-6B	T cells (MOLT 3)	G1/S; G2/M 24, 48 hpi	FACS: tDNA	p53 pathway	[30]
HHV-6A	T cells (SUPT1)	G2/M 24; 48 hpi	FACS: tDNA	Rb modification	[16•]
HHV-6A	T cells (HSB-2)	G2/M 24, 48, 72 hpi	FACS: tDNA	WB of Cyclins	[33]

Abbreviations: exp. v., cells were transfected with expression vector; hpi, hours post infection; WB, western blot analysis; tDNA, total DNA fluorescent staining; V, fluorescent staining of viral components.

checkpoint kinases 1 and 2 (Chk1 and Chk2) can be responsible for the G2/M phase arrest by phosphorylation of CDKs in response to either ssDNA or dsDNA breakage [34,35]. Massive DNA breakage occurs during the cleavage and packaging of viral concatemeric DNA. In addition, in viral infections there is uncoupling between cell cycle phase and the appearance of cellular proteins characteristic of the particular phase [36].

In HHV-6 infections there are uncertainties with regards to the cell cycle arrest at the G2/M phase: first, the cell cycle is defined by measuring total DNA content of the infected cells (Table 1) without considering the viral DNA replication. Second, we expect that the majority of infected cells will be accumulated in the arrested phase. However, in previous reports only moderate increase in the percentage of cells in the G2/M phase were observed. Finally, the infection of HHV-6 leads to fusion of infected cells and formation of syncytia with ballooning shape, causing significant alterations in cell morphology and function.

Summary of the findings and future directions

It was shown that HHV-6 infection of T cells resulted in Rb degradation. The released E2F1 was associated with some cellular transcription as well as transcription of viral genes (Figure 1). The HHV-6 genome encodes a number of genes containing in their promoters the E2F binding site. These include the E2F binding sites in the U27, U74 and U79 promoters which are conserved in the HHV-6A and HHV-6B genomes. A comparison between HHV-6A and HHV-6B regarding the expression of these genes, in primary infection as well as following reactivation from latency, can contribute to the understanding of variation of viral replication in different cell types.

Future studies could involve analyses of additional genes containing the E2F1 binding site in their promoters. Furthermore, the kinetics of synthesis should be followed so as to determine whether the transcription employing E2F binding site follows the regular cascade regulation of immediate early, early and late viral genes expression. An increasing body of evidence demonstrated that HHV-6 induces the accumulation of infected cells at G2/M phase. However, these experiments were based mostly on total DNA analysis. It is necessary to test additional aspects characterizing the phase of the cell cycle as well as the metabolic state of the infected cells. These analyses would potentially increase our understanding of roseolovirus involvement in human diseases and engineering of new antiviral therapeutics.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Polager S, Ginsberg D: **E2F — at the crossroads of life and death**. *Trends Cell Biol* 2008, **18**:528-535.

2. Mundle SD, Saberwal G: **Evolving intricacies and implications of E2F1 regulation**. *FASEB J* 2003, **17**:569-574.

3. Singh S, Johnson J, Chellappan S: **Small molecule regulators of Rb–E2F pathway as modulators of transcription**. *Biochim Biophys Acta* 2010, **1799**:788-794.

4. •• Hume AJ, Kalejta RF: **Regulation of the retinoblastoma proteins by the human herpesviruses**. *Cell Div* 2009, **4**:1.
In-depth review of the alterations in cell cycle during the infections of different human herpesviruses. This review summarizes the data that was found on the matter up to 2009.

5. Song B, Liu JJ, Yeh KC, Knipe DM: **Herpes simplex virus infection blocks events in the G1 phase of the cell cycle**. *Virology* 2000, **267**:326-334.

6. Leisenfelder SA, Moffat JF: **Varicella-zoster virus infection of human foreskin fibroblast cells results in atypical cyclin expression and cyclin-dependent kinase activity**. *J Virol* 2006, **80**:5577-5587.

7. Schang LM, Phillips J, Schaffer PA: **Requirement for cellular cyclin-dependent kinases in herpes simplex virus replication and transcription**. *J Virol* 1998, **72**:5626-5637.

8. Rodriguez A, Jung EJ, Flemington EK: **Cell cycle analysis of Epstein–Barr virus-infected cells following treatment with lytic cycle-inducing agents**. *J Virol* 2001, **75**:4482-4489.

9. An FQ, Compitello N, Horwitz E, Sramkoski M, Knudsen ES, Renne R: **The latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus modulates cellular gene expression and protects lymphoid cells from p16 INK4A-induced cell cycle arrest**. *J Biol Chem* 2005, **280**:3862-3874.

10. Kuny CV, Chinchilla K, Culbertson MR, Kalejta RF: **Cyclin-dependent kinase-like function is shared by the beta- and gamma-subset of the conserved herpesvirus protein kinases**. *PLoS Pathog* 2010, **6**:e1001092.

11. Hume AJ, Finkel JS, Kamil JP, Coen DM, Culbertson MR, Kalejta RF: **Phosphorylation of retinoblastoma protein by viral protein with cyclin-dependent kinase function**. *Science* 2008, **320**:797-799.

12. Kalejta RF: **Functions of human cytomegalovirus tegument proteins prior to immediate early gene expression**. *Curr Top Microbiol Immunol* 2008, **325**:101-115.

13. Kalejta RF: **Tegument proteins of human cytomegalovirus**. *Microbiol Mol Biol Rev* 2008, **72**:249-265.

14. Winkler LL, Kalejta RF: **The 19S proteasome activator promotes human cytomegalovirus immediate early gene expression through proteolytic and non-proteolytic mechanisms**. *J Virol* 2014, **88**:11782-11790.

15. Kalejta RF: **Human cytomegalovirus pp71: a new viral tool to probe the mechanisms of cell cycle progression and oncogenesis controlled by the retinoblastoma family of tumor suppressors**. *J Cell Biochem* 2004, **93**:37-45.

16. • Mlechkovich G, Frenkel N: **Human herpesvirus 6A (HHV-6A) and HHV-6B alter E2F1/Rb pathways and E2F1 localization and cause cell cycle arrest in infected T cells**. *J Virol* 2007, **81**:13499-13508.
First report that showed alterations of the E2F1-Rb pathways during HHV-6A infection, including: a high level of free E2F1, a disassembly of Rb–E2F1 complexes and accumulation of the infected cells in G2/M phase.

17. •• Sharon E, Volchek L, Frenkel N: **Human herpesvirus 6 (HHV-6) alters E2F1/Rb pathways and utilizes the E2F1 transcription factor to express viral genes**. *Proc Natl Acad Sci U S A* 2014, **111**:451-456.

This manuscript reports additional findings regarding the manipulation on E2F1-Rb pathway during HHV-6A infection, showing a Rb degradation, a selective induction of the E2F1 responsive cellular genes, a presence of E2Fs binding sites in HHV-6A genome and utilization of E2F1 and E2Fs binding sites for viral genes expression.

18. Takaku T, Ohyashiki JH, Zhang Y, Ohyashiki K: **Estimating immunoregulatory gene networks in human herpesvirus type 6-infected T cells**. *Biochem Biophys Res Commun* 2005, **336**:469-477.

19. De Bolle L, Hatse S, Verbeken E, De Clercq E, Naesens L: **Human herpesvirus 6 infection arrests cord blood mononuclear cells in G(2) phase of the cell cycle**. *FEBS Lett* 2004, **560**:25-29.

20. Li L, Gu B, Zhou F, Chi J, Feng D, Xie F, Wang F, Ma C, Li M, Wang J *et al.*: **Cell cycle perturbations induced by human herpesvirus 6 infection and their effect on virus replication**. *Arch Virol* 2014, **159**:365-370.

21. Lin K, Ricciardi RP: **The 41-kDa protein of human herpesvirus 6 specifically binds to viral DNA polymerase and greatly increases DNA synthesis**. *Virology* 1998, **250**:210-219.

22. Taniguchi T, Shimamoto T, Isegawa Y, Kondo K, Yamanishi K: **Structure of transcripts and proteins encoded by U79-80 of human herpesvirus 6 and its subcellular localization in infected cells**. *Virology* 2000, **271**:307-320.

23. Bieda M, Xu X, Singer MA, Green R, Farnham PJ: **Unbiased location analysis of E2F1-binding sites suggests a widespread role for E2F1 in the human genome**. *Genome Res* 2006, **16**:595-605.

24. Neill SD, Hemstrom C, Virtanen A, Nevins JR: **An adenovirus E4 gene product trans-activates E2 transcription and stimulates stable E2F binding through a direct association with E2F**. *Proc Natl Acad Sci U S A* 1990, **87**:2008-2012.

25. Workman A, Jones C: **Productive infection and bICP0 early promoter activity of bovine herpesvirus 1 are stimulated by E2F1**. *J Virol* 2010, **84**:6308-6317.

26. Davy C, Doorbar J: **G2/M cell cycle arrest in the life cycle of viruses**. *Virology* 2007, **368**:219-226.

27. Chaurushiya MS, Weitzman MD: **Viral manipulation of DNA repair and cell cycle checkpoints**. *DNA Repair (Amst)* 2009, **8**:1166-1176.

28. Planz O, Pleschka S, Oesterle K, Berberich-Siebelt F, Ehrhardt C, Stitz L, Ludwig S: **Borna disease virus nucleoprotein interacts with the CDC2-cyclin B1 complex**. *J Virol* 2003, **77**:11186-11192.

29. Dietrich J, Blumberg BM, Roshal M, Baker JV, Hurley SD, Mayer-Proschel M, Mock DJ: **Infection with an endemic human herpesvirus disrupts critical glial precursor cell properties**. *J Neurosci* 2004, **24**:4875-4883.

30. Oster B, Bundgaard B, Hollsberg P: **Human herpesvirus 6B induces cell cycle arrest concomitant with p53 phosphorylation and accumulation in T cells**. *J Virol* 2005, **79**:1961-1965.

31. Oster B, Kaspersen MD, Kofod-Olsen E, Bundgaard B, Hollsberg P: **Human herpesvirus 6B inhibits cell proliferation by a p53-independent pathway**. *J Clin Virol* 2006, **37(Suppl. 1)**:S63-S68.

32. • Schleimann MH, Hoberg S, Solhoj Hansen A, Bundgaard B, Witt CT, Kofod-Olsen E, Hollsberg P: **The DR6 protein from human herpesvirus-6B induces p53-independent cell cycle arrest in G2/M**. *Virology* 2014, **452–453**:254-263.
Latest article showing thatthe viral DR6gene induces a cell cycle arrest andaccumulation of treated cells at G2/M phase.

33. Secchiero P, Bertolaso L, Casareto L, Gibellini D, Vitale M, Bemis K, Aleotti A, Capitani S, Franchini G, Gallo RC *et al.*: **Human herpesvirus 7 infection induces profound cell cycle perturbations coupled to disregulation of cdc2 and cyclin B and polyploidization of CD4(+) T cells**. *Blood* 1998, **92**:1685-1696.

34. Li L, Gu B, Zhou F, Chi J, Wang F, Peng G, Xie F, Qing J, Feng D, Lu S *et al.*: **Human herpesvirus 6 suppresses T cell proliferation through induction of cell cycle arrest in infected cells in the G2/M phase**. *J Virol* 2011, **85**:6774-6783.

35. Patil M, Pabla N, Dong Z: **Checkpoint kinase 1 in DNA damage response and cell cycle regulation**. *Cell Mol Life Sci* 2013, **70**:4009-4021.

36. Machida K, Liu JC, McNamara G, Levine A, Duan L, Lai MM: **Hepatitis C virus causes uncoupling of mitotic checkpoint and chromosomal polyploidy through the Rb pathway**. *J Virol* 2009, **83**:12590-12600.

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Current Opinion in Virology

Immune response to HHV-6 and implications for immunotherapy

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Most adults remain chronically infected with HHV-6 after resolution of a primary infection in childhood, with the latent virus held in check by the immune system. Iatrogenic immunosuppression following solid organ transplantation (SOT) or hematopoetic stem cell transplantation (HSCT) can allow latent viruses to reactivate. HHV-6 reactivation has been associated with increased morbidity, graft rejection, and neurological complications post-transplantation. Recent work has identified HHV-6 antigens that are targeted by the CD4+ and CD8+ T cell response in chronically infected adults. T cell populations recognizing these targets can be expanded *in vitro* and are being developed for use in autologous immunotherapy to control post-transplantation HHV-6 reaction.

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Introduction

The increasing clinical importance of HHV-6 demands effective treatment options. Currently, individuals with complicated HHV-6 infection or reactivation are treated with ganciclovir or similar drugs approved for managing other viral infections [1]. However, these drugs have significant toxicity [2] and in some cases are ineffective against HHV-6 [3]. Immunotherapies based on antibodies, expanded T cells, or vaccines potentially could provide an alternative or adjunctive approach to controlling HHV-6 infection. Immunotherapy for human herpesviruses has been in development since the early 1990s [4], and has been shown to be a safe and practical approach to controlling related human herpesviruses human cytomegalovirus (HCMV) [5], Epstein-Barr virus (EBV) [6,7] and herpes simplex virus (HSV) [8]. For HHV-6, little is known about the immune mechanisms that control infection, and current understanding is based largely on a few studies and extrapolation from HCMV [9]. Here we review recent progress in characterizing the immune response to HHV-6 and discuss implications for development of immunotherapies in immunocompromised patients.

Challenges to characterizing the immune response to HHV-6

The lack of a basic understanding of the immune response to HHV-6 has delayed the development of HHV-6 specific immunotherapies. Several aspects of HHV-6 biology interfere with straightforward application of conventional approaches to characterizing antiviral immunity. First, two closely related viruses HHV-6A and HHV-6B have been treated as a single species until very recently [10]. Mounting evidence suggests important differences in the biology of these two viruses and the immune response that they induce [11], but in general they have not been distinguished in studies of the immune response to HHV-6. Second, antibody titers to HHV-6 and frequencies of T cells recognizing HHV-6 are low, making detection of these responses challenging [12••]. Blood samples obtained during active viremia might exhibit higher antibody titers or T cell responses, but symptomatic viremia occurs primarily in young children or immunosuppressed patients from whom sufficient blood samples are difficult to obtain. Third, HHV-6 is a lymphotropic virus that prefers T cells for replication, but also is capable of infecting a variety of antigen presenting cells [1,13]. Profound effects on the normal function of both infected T cells and infected antigen presenting cells have been demonstrated [14–17], and these effects interfere with *ex vivo* analyses. Finally, HHV-6 infection is restricted to humans and closely related primates [18,19], so the lack of a small animal model has inhibited detailed mechanistic studies. Despite these limitations, recently there have been notable advances in defining HHV-6-specific T cell responses and in developing approaches to adoptive immunotherapy.

HHV-6B protective immunity

The observation that primary HHV-6B infection is a mild febrile disease from which most children recover rapidly without complications suggests that protective HHV-6B

immune responses are commonly elicited. After primary infection, HHV-6B is able to persist as a chronic or latent infection controlled by the adaptive immune response. The virus can reactivate under conditions of deficient cell-mediated immunity [20]. Although immunity to HHV-6B could evolve over time, there is evidence that lifelong responses to HHV-6B are imprinted very early after the first onset of HHV-6B infection [21]. Neonates are usually protected from HHV-6B infection by maternal-derived antibodies until titers wane over 3–9 months after birth, making older children susceptible to infection [22]. Primary infection occurs usually before the second year of age, and induces antibodies that persist throughout life [22]. Evidence that T cells are required to control HHV-6B replication is inferred from persistent HHV-6B viral replication in immunosuppressed patients who do not have proliferative T cell responses [20].

Antibody responses

Most studies of the antibody response to HHV-6 have aimed to develop diagnostic methods that differentiate between the three closely related roseoloviruses, HHV-6A, HHV-6B and HHV-7. Little is known about the range of antigens targeted by antibodies recognizing these viruses [23,24]. A few HHV-6 antigens prominently targeted by the antibody response have been identified. These include the major antigenic virion protein U11 [25], the major glycoproteins gH (U48) [26,27] and gQ (U100) [28], the polymerase processivity factor (U27) [29], the late antigen U94 [30], and the tail-anchored membrane protein U24 [31,32] (Figure 1). Serological assays that utilize U11 as antigen are in development for the definition of roseolovirus-specific antibodies. Neutralizing antibodies have been found mainly after primary infection and in transplant recipients but also in some healthy adult donors, which might indicate subclinical reactivation of the virus [33]. Monoclonal neutralizing antibodies targeting gH [34–36], gQ [37] and gB [38] have been described.

T cell responses

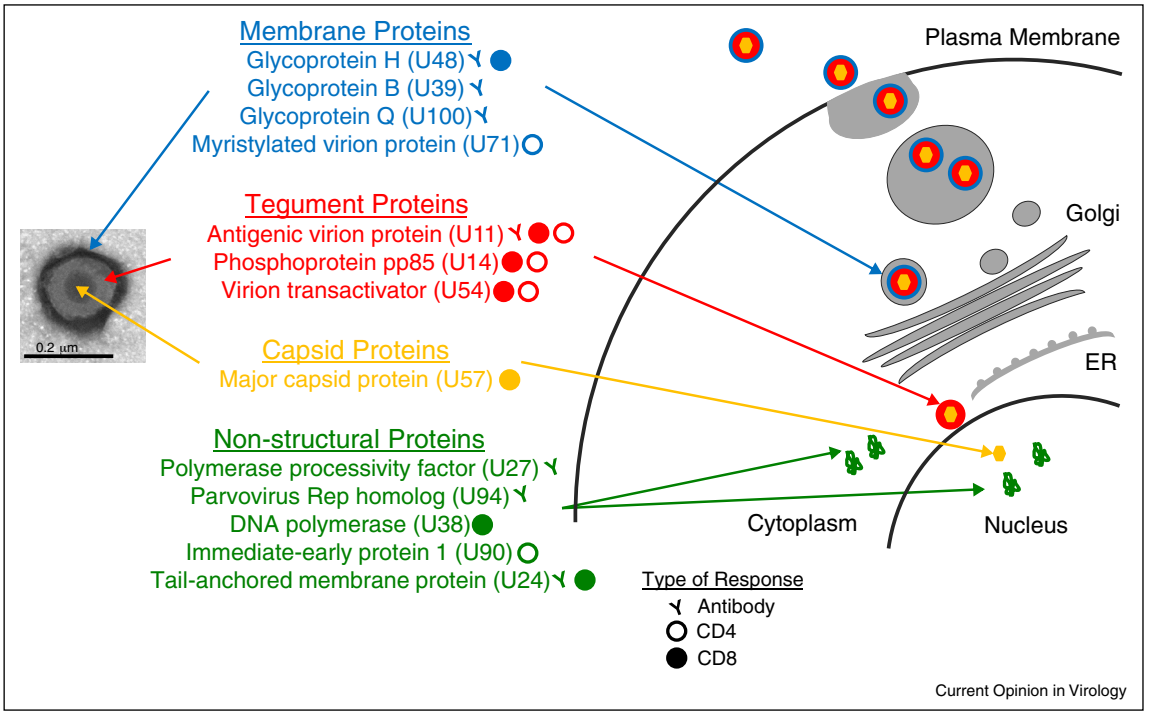
The T cell response to HHV-6 mostly has been characterized using peripheral blood from healthy adults. Responding T cells, measured as the number of IFN-γ producing cells, are present at low frequency (on the order of a few cells per 1000 (or <0.2%) [12••,39••,40••], which contrasts sharply with the stronger responses that are observed for HCMV (up to 4%) [41]. T cell responses have been reported to be higher in HSCT patients [42], but few studies have focused on this population. Despite the low frequency of responding T cells in healthy adults, strong CD4+ and CD8+ T cell proliferative responses are observed [43–45], primarily restricted to memory cells. Proliferation of HHV-6-specific T cells in response to viral antigen allows these low-frequency T cell populations to be expanded *in vitro* for detailed study. The expanded population consists mainly of CD4+ T cells that secrete IFN-γ and exhibit cytotoxic capacity [12••,39••,46]. A prominent subpopulation secretes IL-10 [12••,47•]. IL-4 and low amounts of IL-2 also are also produced [12••], a profile similar to that reported in serum of children with roseola [48••]. The reason for CD4+ T cell skewing and limited CD8+ T cells in expanded populations is not clear. The lower frequency of responding CD8+ T cells in blood ($\sim 10^{-5}$) certainly is a factor [40••], but viral evasion mechanisms also might be responsible.

Shortly after the discovery of HHV-6, the immunosuppressive properties of this virus were recognized. Initially, it was reported that HHV-6 arrests IL-2 synthesis and T cell proliferation [49,50]. Subsequent studies identified immune modulation by effects in both infected and non-infected cells (reviewed in [51]). In infected CD4+ T cells, HHV-6 induces apoptosis [52], inhibition of IL-2 synthesis [14], cell cycle arrest [16], and TCR and MHC-I down modulation [11]. In antigen-presenting cells, HHV-6 induces MHC-I down-modulation [53] and reduces the ability of these cells to present antigens and activate T cells [11]. In addition, IL-10 secreted by CD4+ T cells responding to HHV-6 modulates proliferation of other T cell populations [47•]. Different subsets of regulatory T cells (Tregs) have been observed *in vitro* after HHV-6-specific expansion or cloning [12••,39••,54•].

Targets of the T cell response to HHV-6

The HHV-6 genome encodes ~100 proteins, and many of them are >1000 amino acids in length, making the identification of immunodominant epitopes a laborious and time consuming task. Information on the particular peptide epitopes recognized by T cells is required for identification, characterization, and modulation of T responses specific to HHV-6 as compared to closely related viruses. Approaches used to limit the number of antigens/peptides to screen have focused on HHV-6 proteins present at high levels in virus preparations [12••], or on HHV-6 homologues of antigens defined for HCMV [39••,40••]. Our group used the first approach to define 11 CD4+ T cell epitopes [12••]. These derived from 4 virion proteins (the major capsid protein U57, the tegument proteins U11 and U14, and the glycoprotein U48) and from a non-structural protein (DNA polymerase U38) (Table 1). CD4+ T cells expanded with peptides corresponding to these epitopes responded to APC treated with virus preparations and produced IFN-γ and expressed markers associated with cytotoxic potential. Using the second approach, Martin *et al.* were able to expand CD8+ T cell lines and clones that showed reactivity to peptides from tegument proteins U11 and U54 and showed pro-inflammatory and cytotoxic capabilities [40••]. Also, using the second approach and T cell lines expanded *in vitro*, Gerdemann *et al.* demonstrated T cell responses to peptide epitopes derived from the immediate-early protein U90, the tegument proteins U11, U14

Figure 1



Targets of the adaptive immune response to HHV-6. Antibody and T cell responses target the viral surface membrane, tegument, and capsid components of the virion as well as non-structure proteins expressed in infected cells. Inset (left) shows a purified viral particle with components indicated, and cartoon (right) shows intracellular locations for the expected stepwise viral assembly process.

and U54, and the myristylated virion protein U71 [39••]. The expanded T cells produced IFN-γ and TNF-α and killed antigen-pulsed autologous target cells. Although the expanded T cell populations consisted mainly of CD4+ T cells, epitopes were identified only for the minor component of CD8+ T cells. Additional CD8+ T cell epitopes in U90 were identified recently by Iampietro *et al.* [55]. Overall, twelve CD8+ T cell epitopes were defined: three from U11, two from U54 [40••], six from U90 and one from U14 [39••], to complement the eleven CD4+ T cell epitopes described above. In agreement with earlier reports, many of the mapped T cell responses are crossreactive between HHV-6A and HHV-6B, and so cannot be used as markers of virus-specific responses. Non-crossreactive responses have been reported for capsid antigens, but specific epitopes were not defined [56]. Additionally, three putative HHV-6 epitopes have been defined by virtue of cross-reactivity with human self-antigens (Table 1). T cell responses to a HHV-6 U24 peptide that shares homology with the multiple-sclerosis autoantigen myelin basic protein (MBP) have been reported, but any significance of this crossreactive response in multiple sclerosis is controversial [32,57]. CD4+ T cells recognizing the diabetes-associated glutamic acid decarboxylase islet autoantigen GAD95 were shown to cross-react with a similar peptide sequence from HHV-6A U95 [58], but whether these cells recognize naturally processed antigens is not known. A summary of HHV-6 proteins targeted by CD4+ and CD8+ T cell responses is shown in Figure 1, with specific epitopes listed in Table 1.

HHV-6B reactivation after solid organ transplantation

In the period following immunosuppression, transplant recipients are highly susceptible to common viruses such as herpesviruses, adenoviruses, and seasonal viruses like influenza. HHV-6 reactivation, mostly by HHV-6B [59], occurs in over 40% of HSCT and in up to 60% of solid organ transplant (SOT) recipients [59] during the first weeks after transplantation. HHV-6 reactivation in HSCT patients is associated with graft-versus-host disease, delayed engraftment [60], and CNS dysfunction, including encephalitis that may have long term effects [61•,62•,63••]. In SOT patients, HHV-6B reactivation is associated with prolonged anti-HHV-6 immunosuppression [64], fever, rash, hepatitis, pneumonitis, encephalitis and colitis [59]. In the months after transplantation, T cell proliferative responses to HHV-6 are absent in most individuals. By comparison, proliferative responses to HCMV develop over several weeks (although they do not reach levels observed in healthy donors) [64]. Autologous immune

Table 1

CD4 and CD8 T cell epitopes defined for HHV-6

Epitope	HLA[a]	ORF[b]	Protein	Evidence[c]	Specificity[d]	Reference
GILDFGVKL	A2	U11	Antigenic virion protein	Cyto; grzB	6B	[40••]
MLWYTVYNI	A2	U11	Antigenic virion protein	Cyto; grzB; Tet	6B	[40••]
SLMSGVEPL	A2	U11	Antigenic virion protein	Cyto; grzB	6B	[40••]
ILYGPLTRI	A2	U54	Virion transactivator	CTL; Cyto; grzB; Tet	6B	[40••]
LLCGNLLIL	A2	U54	Virion transactivator	Cyto; grzB	6B	[40••]
TEMMNDARL	B40	U14	Phosphoprotein pp85	CTL; Cyto	n.d.	[39••]
FESLLFPEL	B40	U90	Immediate-early protein 1	CTL; Cyto	n.d.	[39••]
VEESIKEIL	B40	U90	Immediate-early protein 1	CTL; Cyto	n.d.	[39••]
CIQSIGASV	A2	U90	Immediate-early protein 1	CTL; Cyto	6A/6B	[55]
CYAKMLSGK	A3	U90	Immediate-early protein 1	CTL; Cyto	6B/6A?	[55]
STSMFILGK	A3	U90	Immediate-early protein 1	CTL; Cyto	6B/6A?	[55]
NPEISNKEF	B7	U90	Immediate-early protein 1	CTL; Cyto	6B/6A?	[55]
SLESYSASKAFSVPENG	DR1	U11	Antigenic virion protein	Cyto	6A/6B	[12••]
RDNSYMPLIALSLHENG	DR1	U14	Phosphoprotein pp85	Cyto	6A/6B	[12••]
VVGKYSLQDSVLVVRLF	DR1	U38	DNA polymerase	Cyto; Tet	6A/6B	[12••]
GIYYIRVVEVRQMQYDN	DR1	U48	Glycoprotein H	Cyto; Tet	6A/6B	[12••]
VDEEYRFISDATFVDET	DR1	U48	Glycoprotein H	Cyto; Tet	6A/6B	[12••]
TRPLYITMKAQKKNSRI	DR1	U54	Virion transactivator	Cyto; Tet	6A/6B	[12••]
FKSLIYINENTKILEVE	DR1	U57	Major capsid protein	Cyto; Tet	6A/6B	[12••]
IRHHVGIEKPNPSEGEA	DR1	U57	Major capsid protein	Cyto	6A/6B	[12••]
SLLSIMTLAAMHSKLSP	DR1	U57	Major capsid protein	Cyto; Tet	6A/6B	[12••]
TTNPWASLPGSLGDILY	DR1	U57	Major capsid protein	Cyto; Tet	6A/6B	[12••]
DPSRYNISFEALLGIYS	DR1	U57	Major capsid protein	Cyto; Tet	6A/6B	[12••]
KELLQSYVSKNNN	DR53	U95	Immediate-early protein	Cyto	6A?; GAD95	[58]
MDRPRTPPPSYSE	n.d.	U24	Tail-anchored mb. protein	Prolif; Cyto	6A/B; MBP	[32]
RPRTPPPSY	n.d.	U24	Tail-anchored mb. protein	Prolif; CTL	6B?; MBP	[68]

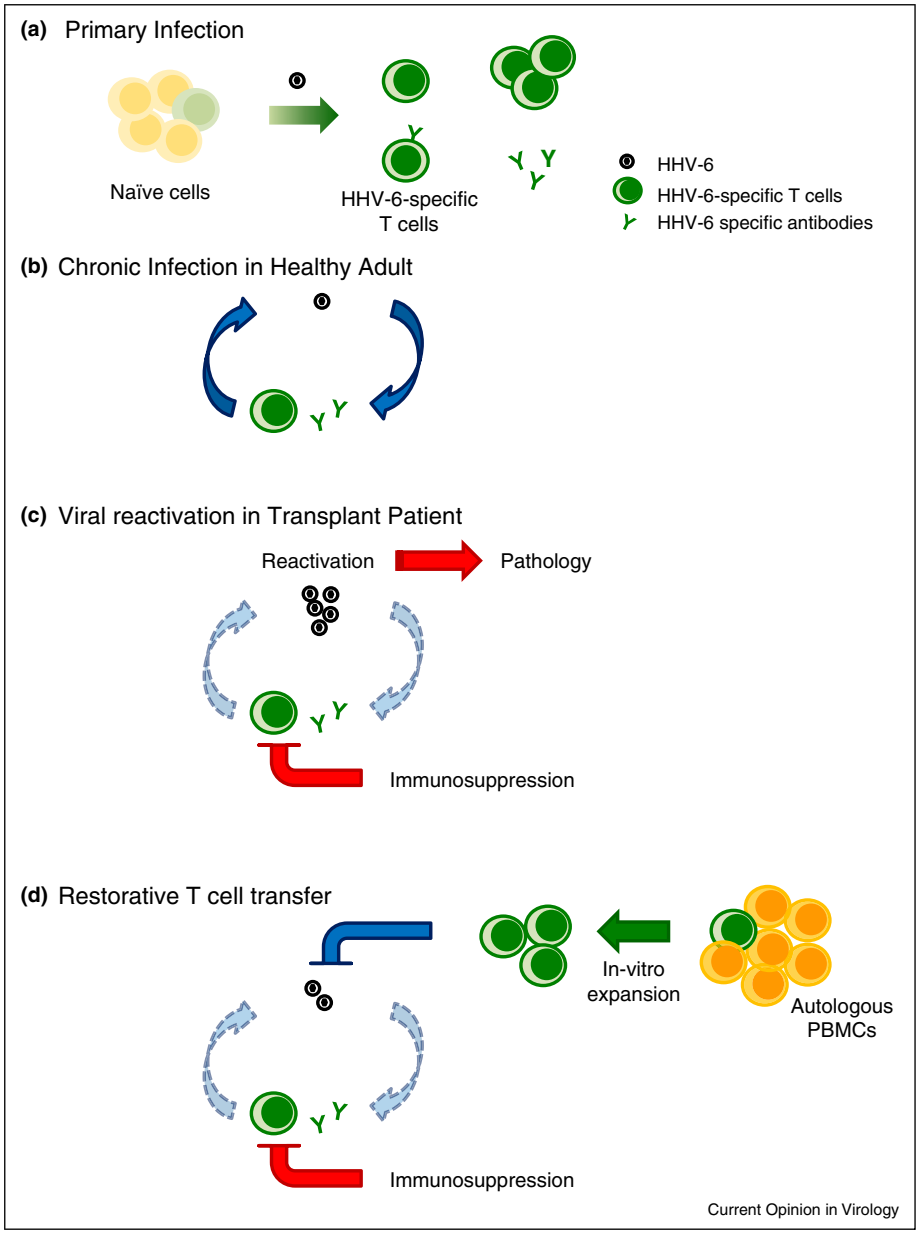
[a] HLA restriction. HLA-A2, HLA-A3, HLA-B7, and HLA-B40 are class I MHC proteins recognized by CD8+ T cells. HLA-DR1 and HLA-DR53 are class II MHC proteins recognized by CD4+ T cells. n.d., not defined.
[b] HHV-6 open reading frame.
[c] CTL, cytotoxicity (cell killing) assay; Cyto, cytokine release; Grzb, granzyme B release; Prolif, cell proliferation assay; Tet, MHC tetramer staining.
[d] Specificity for HHV-6A or HHV-6B, or self antigens, where defined.?, presumptive specificity; n.d. not defined.

enhancement therapy, in which pre-transplantation T cell populations are expanded *ex vivo* and re-introduced (Figure 2), could provide a way to boost the post-transplantation T cell response and help control HHV-6 reactivation.

Autologous immune enhancement therapy

Susceptibility to HHV-6B and other common viruses is associated with deficiency of T cell responses in immunosuppressed individuals [20,65]. Restoration of protective T cell responses against other herpesviruses such as HCMV and EBV has been achieved by transfer of T cells expanded *in vitro* using purified virus, recombinants proteins, or synthetic versions of known immunodominant antigens to stimulate T cell proliferation [5]. A turning point in the immunotherapy to HHV-6B has been the identification of immunodominant antigens and the demonstration that HHV-6B-specific T cells can be expanded in large numbers if cytokines that support T cell expansion are provided in culture [12••,39••,66•]. This knowledge has been rapidly transferred to a small clinical trial that attempted the reconstitution of immune responses to HHV-6B, HCMV, BK virus, EBV, and adenovirus by expansion of PBMCs with a mixture of peptides from these viruses [67••]. The Phase I clinical trial showed that adoptive transfer of peptide-expanded T cells for these viruses was safe, did not induce high levels of cytokines, and did not induce allo-specific responses. Expansion of HHV-6B T cells was performed with overlapping peptides of the immediate early protein 1 (U90) and the tegument proteins U11 and U14 shown to be important targets of the HHV-6B T cell response [12••,39••,40••]. The transferred T cell population consisted of ~60% CD4+ and 35% CD8+ T cells. Although T cell lines were generated from a relatively small number of cells (15 million), and a large number of peptides were included (opening the possibility of peptide competition for binding to MHC proteins), almost 30% of the developed T cell lines had responses to all 5 viruses and 70% to at least 3 viruses. More important and striking was that virus levels were reduced after adoptive transfer of T cells, and this reduction was accompanied by an increase in the number of IFN-γ producing cells. Moreover, three patients that received expanded T cells as prophylaxis were protected from virus reactivation beyond 3 months after the adoptive transfer. However, as indicated by the authors, the clinical trial

Figure 2



Autologous immune enhancement therapy. **(a)** Primary infection with HHV-6 virus elicits antibody and T cell responses. **(b)** During chronic infection virus levels are controlled by antibody and T cell responses. **(c)** In transplantation patients, iatrogenic immunosuppression interferes with immune control of HHV-6 and allows virus reactivation with consequent pathology. **(d)** HHV-6-specific T cells expanded *in vivo* can be reintroduced after transplantation to control virus reactivation.

did not have enough participants to support a claim that reduction in virus levels was a result of infused of T cells rather than other host or viral factors. The T cell population transferred was highly enriched in CD4+ T cells with a minor component of CD8+ T cells, which may have been beneficial since allo-specific disease has been associated with higher frequency of HHV-6-specific CD8+ T cells [42].

Future directions

Although the outcome of the first human HHV-6 immunotherapy by transfer of *in vitro* expanded T cells was favorable, we do not know the epitope specificity of the transferred cells, how the transferred cells contributed to the virus control, or even if the antigens used to expand these cells are the major mediators of protective immune responses. Although a handful of both CD4+ and CD8+ T

cell epitopes have been identified, in most cases these were identified by reference to homologous HCMV antigens, and it is not clear how broadly these represent the overall HHV-6-specific response. A better understanding of the repertoire of peptides recognized by HHV-6-specific T cells would provide additional possibilities for *in vitro* T cell expansion for adoptive immunotherapy. In general studies of HHV-6 T cell epitopes have been performed using blood samples from healthy but chronically infected adults. Characterization of the T cell responses induced by primary infection in childhood, or by virus reactivation in transplantation patients where viremia is controlled, would be helpful to identify protective epitopes. Experiments using *in vitro* expanded T cell populations have identified both productive and suppressive responses, but these have not been associated with particular epitopes. Several *in vitro* studies have suggested that IL-10 produced by CD4+ T cells could play a major role in limiting the expansion of both CD4+ and CD8+ T cell responses. A better understanding of various types and functions of T cells recognizing HHV-6 antigens might allow beneficial responses to be preferentially expanded. New adoptive immunotherapy protocols incorporating this information might allow protection to be obtained with a lower number of transferred cells, limiting the time and resources needed for expansion and reducing the possibility of expansion of unwanted cell responses.

Conclusions

Important advances in defining the T cell response to HHV-6 have allowed the first clinical trial in HHV-6 immunotherapy. Although responses to HHV-6 antigens are present in PBMCs at low frequency, CD4+ and CD8+ T cell antigens have been identified from a variety of viral proteins. Sufficient numbers of cells for immunotherapies can be generated if cells are expanded in medium containing antigen and cytokines. Promising results from a clinical trial reported a decrease in virus load in patients with HHV-6 reactivation after transfer of *in vitro* expanded T cells, suggesting that immunotherapies for HHV-6 are possible without large numbers or antigenic specificities of responding cells. Whether the observed reduction in virus load was mediated by CD4+ or CD8+ T cells and the epitope specificity of these responses remains unknown. Since HHV-6 T cell reactivity has been associated with multiple sclerosis and other autoimmune diseases, future studies should distinguish protective and allo-specific epitopes to minimize potentially cross-reactive autoimmune responses. Nevertheless, efforts to improve the efficacy of HHV-6 therapies will greatly benefit the populations at risk of severe viral disease.

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References and recommended reading

Papers of particular interest, published within the period of review,

have been highlighted as:

- • of special interest
- •• of outstanding interest

1. De Bolle L, Naesens L, De Clercq E: **Update on human herpesvirus 6 biology, clinical features, and therapy**. *Clin Microbiol Rev* 2005, **18**:217-245.

2. Biron KK: **Antiviral drugs for cytomegalovirus diseases**. *Antiviral Res* 2006, **71**:154-163.

3. Zerr DM, Gupta D, Huang ML, Carter R, Corey L: **Effect of antivirals on human herpesvirus 6 replication in hematopoietic stem cell transplant recipients**. *Clin Infect Dis* 2002, **34**:309-317.

4. Riddell SR, Watanabe KS, Goodrich JM, Li CR, Agha ME, Greenberg PD: **Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones**. *Science* 1992, **257**:238-241.

5. Hanley PJ, Bollard CM: **Controlling cytomegalovirus: helping the immune system take the lead**. *Viruses* 2014, **6**:2242-2258.

6. Moosmann A, Bigalke I, Tischer J, Schirrmann L, Kasten J, Tippmer S, Leeping M, Prevalsek D, Jaeger G, Ledderose G *et al.*: **Effective and long-term control of EBV PTLD after transfer of peptide-selected T cells**. *Blood* 2010, **115**:2960-2970.

7. Icheva V, Kayser S, Wolff D, Tuve S, Kyzirakos C, Bethge W, Greil J, Albert MH, Schwinger W, Nathrath M *et al.*: **Adoptive transfer of epstein-barr virus (EBV) nuclear antigen 1-specific t cells as treatment for EBV reactivation and lymphoproliferative disorders after allogeneic stem-cell transplantation**. *J Clin Oncol* 2013, **31**:39-48.

8. Belshe RB, Leone PA, Bernstein DI, Wald A, Levin MJ, Stapleton JT, Gorfinkel I, Morrow RL, Ewell MG, Stokes-Riner A *et al.*: **Efficacy results of a trial of a herpes simplex vaccine**. *N Engl J Med* 2012, **366**:34-43.

9. Wang FZ, Pellett PE: **HHV-6A, 6B, and 7: immunobiology and host response**. In *Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis*. Edited by Arvin A, Campadelli-Fiume G, Mocarski E, Moore PS, Roizman B, Whitley R, Yamanishi K. Cambridge, UK: Cambridge University Press; 2007.

10. Ablashi D, Agut H, Alvarez-Lafuente R, Clark DA, Dewhurst S, DiLuca D, Flamand L, Frenkel N, Gallo R, Gompels UA *et al.*: **Classification of HHV-6A and HHV-6B as distinct viruses**. *Arch Virol* 2014, **159**:863-870.

11. Dagna L, Pritchett JC, Lusso P: **Immunomodulation and immunosuppression by human herpesvirus 6A and 6B**. *Future Virol* 2013, **8**:273-287.

12. •• Nastke MD, Becerra A, Yin L, Dominguez-Amorocho O, Gibson L, Stern LJ, Calvo-Calle JM: **Human CD4+ T cell response to human herpesvirus 6**. *J Virol* 2012, **86**:4776-4792.
Report of the first antigen-specific CD4 T cell epitopes from HHV-6, and characterization of the cytokine response to HHV-6 in healthy adults. Development of HLA-DR1 tetramers for the study of CD4+ T cell responses to HHV-6.

13. Takahashi K, Sonoda S, Higashi K, Kondo T, Takahashi H, Takahashi M, Yamanishi K: **Predominant CD4 T-lymphocyte tropism of human herpesvirus 6-related virus**. *J Virol* 1989, **63**:3161-3163.

14. Iampietro M, Morissette G, Gravel A, Flamand L: **Inhibition of interleukin-2 gene expression by human herpesvirus 6B U54 tegument protein**. *J Virol* 2014, **88**:12452-12463.

15. Gupta S, Agrawal S, Gollapudi S: **Differential effect of human herpesvirus 6A on cell division and apoptosis among naive and central and effector memory CD4+ and CD8+ T-cell subsets**. *J Virol* 2009, **83**:5442-5450.

16. Li L, Gu B, Zhou F, Chi J, Wang F, Peng G, Xie F, Qing J, Feng D, Lu S *et al.*: **Human herpesvirus 6 suppresses T cell proliferation through induction of cell cycle arrest in infected cells in the G2/M phase**. *J Virol* 2011, **85**:6774-6783.

17. Smith AP, Paolucci C, Di Lullo G, Burastero SE, Santoro F, Lusso P: **Viral replication-independent blockade of dendritic cell maturation and interleukin-12 production by human herpesvirus 6**. *J Virol* 2005, **79**:2807-2813.

18. Santoro F, Kennedy PE, Locatelli G, Malnati MS, Berger EA, Lusso P: **CD46 is a cellular receptor for human herpesvirus 6**. *Cell* 1999, **99**:817-827.

19. Leibovitch E, Wohler JE, Cummings Macri SM, Motanic K, Harberts E, Gaitan MI, Maggi P, Ellis M, Westmoreland S, Silva A *et al.*: **Novel marmoset (Callithrix jacchus) model of human Herpesvirus 6A and 6B infections: immunologic, virologic and radiologic characterization**. *PLoS Pathog* 2013, **9**:e1003138.

20. Wang FZ, Linde A, Dahl H, Ljungman P: **Human herpesvirus 6 infection inhibits specific lymphocyte proliferation responses and is related to lymphocytopenia after allogeneic stem cell transplantation**. *Bone Marrow transplant* 1999, **24**:1201-1206.

21. Koide W, Ito M, Torigoe S, Ihara T, Kamiya H, Sakurai M: **Activation of lymphocytes by HHV-6 antigen in normal children and adults**. *Viral Immunol* 1998, **11**:19-25.

22. Okuno T, Takahashi K, Balachandra K, Shiraki K, Yamanishi K, Takahashi M, Baba K: **Seroepidemiology of human herpesvirus 6 infection in normal children and adults**. *J Clin Microbiol* 1989, **27**:651-653.

23. Balachandran N, Amelse RE, Zhou WW, Chang CK: **Identification of proteins specific for human herpesvirus 6-infected human T cells**. *J Virol* 1989, **63**:2835-2840.

24. Yamamoto M, Black JB, Stewart JA, Lopez C, Pellett PE: **Identification of a nucleocapsid protein as a specific serological marker of human herpesvirus 6 infection**. *J Clin Microbiol* 1990, **28**:1957-1962.

25. Pellett PE, Sanchez-Martinez D, Dominguez G, Black JB, Anton E, Greenamoyer C, Dambaugh TR: **A strongly immunoreactive virion protein of human herpesvirus 6 variant B strain Z29: identification and characterization of the gene and mapping of a variant-specific monoclonal antibody reactive epitope**. *Virology* 1993, **195**:521-531.

26. Qian G, Wood C, Chandran B: **Identification and characterization of glycoprotein gH of human herpesvirus-6**. *Virology* 1993, **194**:380-386.

27. Anderson RA, Gompels UA: **N- and C-terminal external domains of human herpesvirus-6 glycoprotein H affect a fusion-associated conformation mediated by glycoprotein L binding the N terminus**. *J Gen Virol* 1999, **80**:1485-1494.

28. Pfeiffer B, Berneman ZN, Neipel F, Chang CK, Tirwatnapong S, Chandran B: **Identification and mapping of the gene encoding the glycoprotein complex gp82-gp105 of human herpesvirus 6 and mapping of the neutralizing epitope recognized by monoclonal antibodies**. *J Virol* 1993, **67**:4611-4620.

29. Chang CK, Balachandran N: **Identification, characterization, and sequence analysis of a cDNA encoding a phosphoprotein of human herpesvirus 6**. *J Virol* 1991, **65**:7085.

30. Caselli E, Boni M, Bracci A, Rotola A, Cermelli C, Castellazzi M, Di Luca D, Cassai E: **Detection of antibodies directed against human herpesvirus 6 U94/REP in sera of patients affected by multiple sclerosis**. *J Clin Microbiol* 2002, **40**:4131-4137.

31. Soldan SS, Berti R, Salem N, Secchiero P, Flamand L, Calabresi PA, Brennan MB, Maloni HW, McFarland HF, Lin HC *et al.*: **Association of human herpes virus 6 (HHV-6) with multiple sclerosis: increased IgM response to HHV-6 early antigen and detection of serum HHV-6 DNA**. *Nat Med* 1997, **3**:1394-1397.

32. Tejada-Simon MV, Zang YC, Hong J, Rivera VM, Zhang JZ: **Cross-reactivity with myelin basic protein and human herpesvirus-6 in multiple sclerosis**. *Ann Neurol* 2003, **53**:189-197.

33. Suga S, Yoshikawa T, Asano Y, Nakashima T, Yazaki T, Fukuda M, Kojima S, Matsuyama T, Ono Y, Oshima S: **IgM neutralizing antibody responses to human herpesvirus-6 in patients with exanthem subitum or organ transplantation**. *Microbiol Immunol* 1992, **36**:495-506.

34. Foa-Tomasi L, Boscaro A, di Gaeta S, Campadelli-Fiume G: **Monoclonal antibodies to gp100 inhibit penetration of human herpesvirus 6 and polykaryocyte formation in susceptible cells**. *J Virol* 1991, **65**:4124-4129.

35. Liu DX, Gompels UA, Foa-Tomasi L, Campadelli-Fiume G: **Human herpesvirus-6 glycoprotein H and L homologs are components of the gp100 complex and the gH external domain is the target for neutralizing monoclonal antibodies**. *Virology* 1993, **197**:12-22.

36. Takeda K, Haque M, Sunagawa T, Okuno T, Isegawa Y, Yamanishi K: **Identification of a variant B-specific neutralizing epitope on glycoprotein H of human herpesvirus-6**. *J Gen Virol* 1997, **78**:2171-2178.

37. Kawabata A, Oyaizu H, Maeki T, Tang H, Yamanishi K, Mori Y: **Analysis of a neutralizing antibody for human herpesvirus 6B reveals a role for glycoprotein Q1 in viral entry**. *J Virol* 2011, **85**:12962-12971.

38. Foa-Tomasi L, Guerrini S, Huang T, Campadelli-Fiume G: **Characterization of human herpesvirus-6(U1102) and (GS) gp112 and identification of the Z29-specified homolog**. *Virology* 1992, **191**:511-516.

39. •• Gerdemann U, Keukens L, Keirnan JM, Katari UL, Nguyen CT, de Pagter AP, Ramos CA, Kennedy-Nasser A, Gottschalk SM, Heslop HE *et al.*: **Immunotherapeutic strategies to prevent and treat human herpesvirus 6 reactivation after allogeneic stem cell transplantation**. *Blood* 2013, **121**:207-218.

Report of additional CD8+ T cell epitope restricted by HLA-B*40 HHV-6 T cell epitopes, *ex vivo* expansion of HHV-6 specific cytotoxic CD8+ T cells and suggestions that these could be used in immunotherapy.

40. •• Martin LK, Schub A, Dillinger S, Moosmann A: **Specific CD8(+) T cells recognize human herpesvirus 6B**. *Eur J Immunol* 2012, **42**:2901-2912.

Report of the first antigen-specific CD8+ T cell epitope from HHV-6, and observation of a low frequency of HHV-6 antigen-specific CD8+ T cells recognizing these epitopes in healthy adults. Development of HLA-A2 tetramers for study of HLA-A2 T cell responses to HHV-6.

41. Sylwester AW, Mitchell BL, Edgar JB, Taormina C, Pelte C, Ruchti F, Sleath PR, Grabstein KH, Hosken NA, Kern F *et al.*: **Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects**. *J Exp Med* 2005, **202**:673-685.

42. de Pagter AP, Boelens JJ, Scherrenburg J, Vroom-de Blank T, Tesselaar K, Nanlohy N, Sanders EA, Schuurman R, van Baarle D: **First analysis of human herpesvirus 6T-cell responses: specific boosting after HHV6 reactivation in stem cell transplantation recipients**. *Clin Immunol* 2012, **144**:179-189.

43. Yakushijin Y, Yasukawa M, Kobayashi Y: **T-cell immune response to human herpesvirus-6 in healthy adults**. *Microbiol Immunol* 1991, **35**:655-660.

44. Tejada-Simon MV, Zang YC, Hong J, Rivera VM, Killian JM, Zhang JZ: **Detection of viral DNA and immune responses to the human herpesvirus 6 101-kilodalton virion protein in patients with multiple sclerosis and in controls**. *J Virol* 2002, **76**:6147-6154.

45. Haveman LM, Scherrenburg J, Maarschalk-Ellerbroek LJ, Hoek PD, Schuurman R, de Jager W, Ellerbroek PM, Prakken BJ, van Baarle D, van Montfrans JM: **T-cell response to viral antigens in adults and children with common variable immunodeficiency and specific antibody deficiency**. *Clin Exp Immunol* 2010, **161**:108-117.

46. Yakushijin Y, Yasukawa M, Kobayashi Y: **Establishment and functional characterization of human herpesvirus 6-specific CD4+ human T-cell clones**. *J Virol* 1992, **66**:2773-2779.

47. • Wang F, Yao K, Yin QZ, Zhou F, Ding CL, Peng GY, Xu J, Chen Y, Feng DJ, Ma CL *et al.*: **Human herpesvirus-6-specific interleukin 10-producing CD4+ T cells suppress the CD4+ T-cell response in infected individuals**. *Microbiol Immunol* 2006, **50**:787-803.

Report showing that *in vitro* expanded HHV-6-specific T cells secrete high levels of IL-10, which inhibits CD4 and CD8 T cell responses.

48. •• Yoshikawa T, Kato Y, Ihira M, Nishimura N, Ozaki T, Kumagai T, Asano Y: **Kinetics of cytokine and chemokine responses in**

patients with primary human herpesvirus 6 infection. *J Clin Virol* 2011, **50**:65-68.
Unique analysis of the cytokine and chemokine response to HHV-6 in childeren suffering from roseola.

49. Flamand L, Gosselin J, Stefanescu I, Ablashi D, Menezes J: **Immunosuppressive effect of human herpesvirus 6 on T-cell functions: suppression of interleukin-2 synthesis and cell proliferation**. *Blood* 1995, **85**:1263-1271.

50. Horvat RT, Parmely MJ, Chandran B: **Human herpesvirus 6 inhibits the proliferative responses of human peripheral blood mononuclear cells**. *J Infect Dis* 1993, **167**:1274-1280.

51. Lusso P: **HHV-6 and the immune system: mechanisms of immunomodulation and viral escape**. *J Clin Virol* 2006, **37**:S4-S10.

52. Furukawa M, Yasukawa M, Yakushijin Y, Fujita S: **Distinct effects of human herpesvirus 6 and human herpesvirus 7 on surface molecule expression and function of CD4+ T cells**. *J Immunol* 1994, **152**:5768-5775.

53. Glosson NL, Hudson AW: **Human herpesvirus-6A and -6B encode viral immunoevasins that downregulate class I MHC molecules**. *Virology* 2007, **365**:125-135.

54. • Wang F, Chi J, Peng G, Zhou F, Wang J, Li L, Feng D, Xie F, Gu B, Qin J *et al.*: **Development of virus-specific CD4+ and CD8+ regulatory T cells induced by human herpesvirus 6 infection**. *J Virol* 2014, **88**:1011-1024.
First report of classical regulatory T cells recognizing HHV-6.

55. Iampietro M, Morissette G, Gravel A, Dubuc I, Rousseau M, Hasan A, O'Reilly RJ, Flamand L: **Human herpesvirus 6B immediate early I protein contains functional HLA-A*02, A*03 and B*07 class I-restricted CD8 T cell epitopes**. *Eur J Immunol* 2014. In Press.

56. Wang FZ, Dahl H, Ljungman P, Linde A: **Lymphoproliferative responses to human herpesvirus-6 variant A and variant B in healthy adults**. *J Med Virol* 1999, **57**:134-139.

57. Cirone M, Cuomo L, Zompetta C, Ruggieri S, Frati L, Faggioni A, Ragona G: **Human herpesvirus 6 and multiple sclerosis: a study of T cell cross-reactivity to viral and myelin basic protein antigens**. *J Med Virol* 2002, **68**:268-272.

58. Uemura Y, Senju S, Maenaka K, Iwai LK, Fujii S, Tabata H, Tsukamoto H, Hirata S, Chen YZ, Nishimura Y: **Systematic analysis of the combinatorial nature of epitopes recognized by TCR leads to identification of mimicry epitopes for glutamic acid decarboxylase 65-specific TCRs**. *J Immunol* 2003, **170**:947-960.

59. Razonable RR, Zerr DM: **HHV-6, HHV-7 and HHV-8 in solid organ transplant recipients**. *Am J Transpl* 2009, **9**:S100-S103.

60. Ablashi DV, Devin CL, Yoshikawa T, Lautenschlager I, Luppi M, Kuhl U, Komaroff AL: **Review part 3: human herpesvirus-6 in multiple non-neurological diseases**. *J Med Virol* 2010, **82**:1903-1910.

61. • Ogata M, Satou T, Kadota J, Saito N, Yoshida T, Okumura H, Ueki T, Nagafuji K, Kako S, Uoshima N *et al.*: **Human herpesvirus 6 (HHV-6) reactivation and HHV-6 encephalitis after allogeneic hematopoietic cell transplantation: a multicenter, prospective study**. *Clin Infect Dis* 2013, **57**:671-681.

62. • Scheurer ME, Pritchett JC, Amirian ES, Zemke NR, Lusso P, Ljungman P: **HHV-6 encephalitis in umbilical cord blood transplantation: a systematic review and meta-analysis**. *Bone Marrow Transpl* 2013, **48**:574-580.
These two papers show that encephalitis is a major complication in HSCT transplantation.

63. •• Zerr DM, Fann JR, Breiger D, Boeckh M, Adler AL, Xie H, Delaney C, Huang ML, Corey L, Leisenring WM: **HHV-6 reactivation and its effect on delirium and cognitive functioning in hematopoietic cell transplantation recipients**. *Blood* 2011, **117**:5243-5249.
This prospective study of CNS dysfunction in hematopoietic stem cell transplantation patients shows an association between HHV-6 reaction and neurocognitive decline in the first 3 months following the transplant.

64. Singh N, Bentlejewski C, Carrigan DR, Gayowski T, Knox KK, Zeevi A: **Persistent lack of human herpesvirus-6 specific T-helper cell response in liver transplant recipients**. *Transpl Infect Dis* 2002, **4**:59-63.

65. de Pagter PJ, Boelens JJ, Jacobi R, Schuurman R, Nanlohy NM, Sanders EA, van Baarle D: **Increased proportion of perforin-expressing CD8+ T-cells indicates control of herpesvirus reactivation in children after stem cell transplantation**. *Clin Immunol* 2013, **148**:92-98.

66. • Gerdemann U, Keirnan JM, Katari UL, Yanagisawa R, Christin AS, Huye LE, Perna SK, Ennamuri S, Gottschalk S, Brenner MK *et al.*: **Rapidly generated multivirus-specific cytotoxic T lymphocytes for the prophylaxis and treatment of viral infections**. *Mol Ther* 2012, **20**:1622-1632.
Protocol for the generation of multivirus-specific CTL, to 7 viruses including HHV-6, CMV, EBV, adenovirus, BK, RSV and flu is presented and is the basis for the clinical trial reported by Papadopoulou 2014.

67. •• Papadopoulou A, Gerdemann U, Katari UL, Tzannou I, Liu H, Martinez C, Leung K, Carrum G, Gee AP, Vera JF *et al.*: **Activity of broad-spectrum T cells as treatment for AdV, EBV, CMV, BKV, and HHV6 infections after HSCT**. *Sci Transl Med* 2014, **6**:242ra283.
First report of HHV-6 immunotherapy in HSCT patients in a small clinical trial. Virus load was controlled by transfer of *in vitro* expanded T cells.

68. Cheng W, Ma Y, Gong F, Hu C, Qian L, Huang Q, Yu Q, Zhang J, Chen S, Liu Z *et al.*: **Cross-reactivity of autoreactive T cells with MBP and viral antigens in patients with MS**. *Front Biosci* 2012, **17**:1648-1658.

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Current Opinion in Virology

The development of new therapies for human herpesvirus 6

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Human herpesvirus 6 (HHV-6) infections are typically mild and in rare cases can result in encephalitis. A common theme among all the herpesviruses, however, is the reactivation upon immune suppression. HHV-6 commonly reactivates in transplant recipients. No therapies are approved currently for the treatment of these infections, although small studies and individual case reports have reported intermittent success with drugs such as cidofovir, ganciclovir, and foscarnet. In addition to the current experimental therapies, many other compounds have been reported to inhibit HHV-6 in cell culture with varying degrees of efficacy. Recent advances in the development of new small molecule inhibitors of HHV-6 will be reviewed with regard to their efficacy and spectrum of antiviral activity. The potential for new therapies for HHV-6 infections will also be discussed, and they will likely arise from efforts to develop broad spectrum antiviral therapies for DNA viruses.

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Introduction

Human herpesvirus 6 A (HHV-6A) and human herpesvirus 6 B (HHV-6B) are members of the betaherpesvirus subfamily, as is cytomegalovirus (CMV) and Human Herpesvirus 7 (HHV-7). Primary infections with the Roseoloviruses HHV-6A and HHV-6B typically occur early in life with HHV-6B being the most common [1]. The two HHV-6 viruses are distinct entities and are classified as different species [2,3]; they are associated with different clinical manifestations, yet it is not always feasible to distinguish the viruses in clinical studies so data from viruses are generally analyzed together and reported simply as HHV-6 infections. Here, we will specify the specific virus where possible and will simply use the HHV-6 designation where it is not. Primary infection with HHV-6B has been shown to be the cause of exanthem subitum (roseola) in infants [4], and can also result in an infectious mononucleosis-like illness in adults [5]. Infections caused by HHV-6A and HHV-7 have not been well characterized and are typically reported in the transplant setting [6,7]. Serologic studies indicated that most people become infected with HHV-6 by the age of two, most likely through saliva transmission [8]. The receptors for HHV-6A and HHV-6B have been identified as CD46 and CD134, respectively [9,10]. This facilitates entry into many cell types including CD4+ cells, CD8+ T-cells, natural killer cells, monocytes, epithelial cells, and brain-derived cells [11].

The development of therapies for HHV-6 infections has been limited because of the lack of unequivocal association between infection and disease that warrants intervention. Roseola infections are typically mild and do not warrant therapy. Other infections caused by HHV-6 have been incriminated in a variety of human illnesses; however, the lack of cause and effect has impeded drug development and controlled studies of existing medications in order to establish value of treatment are wanting. In large part, such associations may be dependent upon controlled clinical trials that establish the value of therapies in targeted diseases. Diseases associated with HHV-6 infection have included encephalitis and infections in immunocompromised host, particularly interstitial pneumonitis [12]. In addition, infection has been incriminated as a cause of multiple sclerosis, as has been the case for numerous other viral agents [13]. Reactivation of HHV-6 frequently occurs during immune suppression and is seen in 50% of all bone marrow and 20–30% of solid organ transplant recipients [7,14]. Two clinical studies suggest a role of HHV-6 in contributing to morbidity in hematopoietic stem cell transplant recipients. Specifically the early reactivation with increasing viral load was associated with fever, skin rash, diarrhea, pulmonary complications, and neurologic disorders [15]. A second study utilized prophylactic ganciclovir in a placebo-controlled study that demonstrated drug administration decreased the probability of skin rash, interstitial pneumonitis, diarrhea, and thrombatic microangiopathy (TMA) [16]. Diagnostic procedures and sequencing analyses have shown that the viral genome can integrate within telomeric regions of chromosomes in some individuals, although

its significance remains unclear [17,18•]. While these studies do not define disease etiology, they provide potential indications for the development of therapeutics for antiviral agents.

Methodologic assays

Several methodologies have been employed to detect activity of small molecules against all of the betaherpesviruses and will be summarized specifically for HHV-6 infection. All isolates of HHV-6A and HHV-6B replicate well in phytohemagglutinin-stimulated umbilical cord blood lymphocytes and exhibit a prolonged replication cycle characteristic of this subfamily [19]. Additional cell lines that support viral replication have been identified and are generally used in the evaluation of antiviral activity. The first report of antiviral activity against GS strain of HHV-6A was described in a T-lymphoblastoid cell line (HSB-2) [20]. The Z29 strain of HHV-6B replicates well in Molt-3 cells, and this line is most often used in evaluating the efficacy of antiviral agents [21]. In all cells, viral replication can be assessed by DNA hybridization, quantitative PCR, and flow cytometry, but cytopathology is also apparent in some cell lines [20,22•,23].

Molecules with antiviral activity against HHV-6

The susceptibility of HHV-6 to antiviral drugs seems to be distinct from that of CMV, although CDV, PFA, and GCV all appear to inhibit virus replication *in vitro* with modest efficacy [20,24]. To summarize the activity of the more commonly used compounds, the *in vitro* efficacy from several manuscripts is shown in Table 1. Additional effective agents are in various stages of development and the most promising small molecules will be discussed in detail below (Figure 1). Immunotherapeutic strategies have also been reported for the therapy of HHV-6 infections but are outside the scope of this review [25].

Ganciclovir

The one molecule studied extensively for therapy of putative HHV-6 infection is ganciclovir, a nucleoside analog that was synthesized in the 1980s for the treatment of CMV infection. This compound is phosphorylated by the U69 kinase in HHV-6 and the active triphosphate metabolite inhibits the DNA polymerase. The activity of ganciclovir *in vitro* is dependent upon the assay that has been employed. Utilizing an immunofluorescence assay, ganciclovir was reported to have a minimal level of *in vitro* activity at >25 μM [20]. However, in a more sensitive assay that utilized cord blood lymphocytes, Yoshida and colleagues reported activity in the μM range [26]. In large part, this limited activity against both viruses may be related to the low level of phosphorylation by the U69 kinase, and, as a consequence, the reduced inhibition of DNA polymerase by the active metabolite [27,28]. Resistance to the drug maps both the U69 protein kinase as well as the U38 DNA polymerase, and the mechanism of action is thought to be similar to that against CMV [29,30].

Several clinical trials have suggested the value of ganciclovir, but from a very limited perspective [16,31–37]. Of note, resistant virus has been detected in a number of transplant patients and is not unexpected given the modest efficacy of ganciclovir against this virus [38••,39].

With the development of the oral formulation of ganciclovir, valganciclovir, an alternative to intravenous therapy exists and provides an opportunity for the performance of controlled clinical trials with greater ease of drug administration in those populations that tolerate orally administered medications.

Foscarnet

Foscarnet is employed to treat CMV infections in the immunocompromised host, particularly in the presence of antiviral resistance to ganciclovir. This drug directly binds the pyrophosphate binding site in the DNA polymerase and inhibits the activity of this enzyme. It was among the first drugs identified to have activity against HHV-6 replication *in vitro* at a level of approximately 25–50 μM, and it is active against both viruses [20,40]. In clinical studies the drug has been used alone and with other licensed drugs, including ganciclovir and cidofovir for the treatment of HHV-6 infections [33,35,37,41,42]. Notably, the electrolyte imbalances resulting from therapy result in renal toxicity that is a deterrent to its use. As would be anticipated from

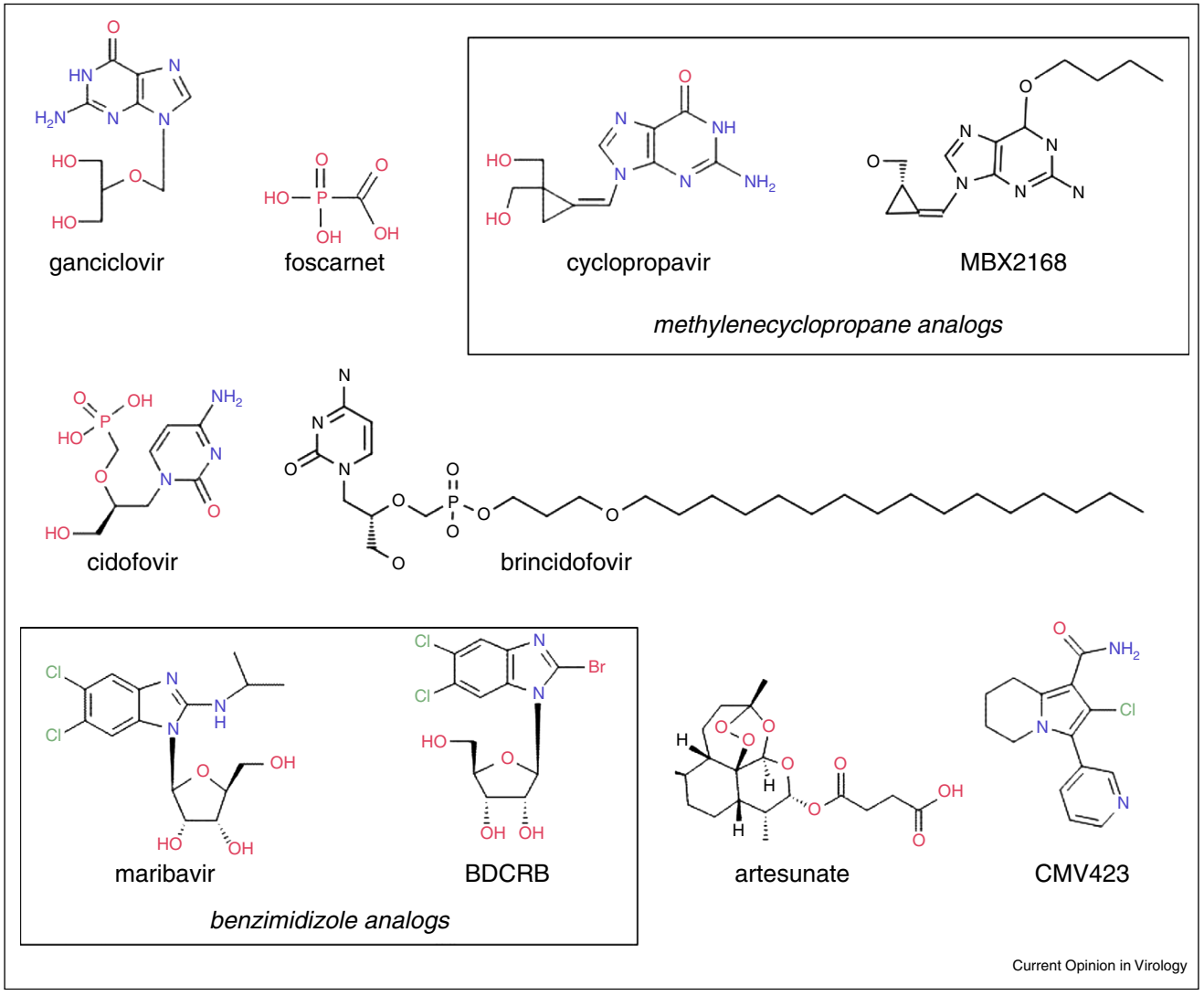
Table 1

Efficacy of selected antiviral drugs against HHV-6A and HHV-6B

	EC_{50} for HHV-6A (μM)[a]		EC_{50} for HHV6B (μM)		Ref
	Mean[a]	Range	Mean	Range	
foscarnet	23.4	6.7–53	50	22–86	[20,26,68]
cidofovir	4.72	0.33–14	6.5	2.3–13	[20,22•,26,47,53,60,68]
cyclic cidofovir	2.9	0.79–6.3	9	5.4–16	[20,26,47]
ganciclovir	17	2.0–25	4.5	NA	[20,26]
cyclopropavir	4.5	1.3–7	1.6	0.7–2.5	[22•,53]
brincidofovir	0.003	NA	0.007	NA	[47]
L BDCRB	2.8	NA	9.7	NA	[60]

[a] The mean of EC_{50} values are shown from the indicated publications although not all of them report the efficacy of both HHV-6A and HHV-6B.

Figure 1



Structures of selected compounds with antiviral activity against HHV-6. Structures for selected compounds with antiviral activity. Classes of specific compounds are designated in boxes. The structure for D BDCRB is shown although both the L and D isomers are discussed.

its mechanism of action, resistance to foscarnet maps to the DNA polymerase [43].

Cidofovir

Cidofovir is licensed for the therapy of CMV infections, particularly in high-risk immunocompromised hosts. Historically, the medication was used as an alternative treatment to ganciclovir in AIDS patients with retinitis. This acyclic nucleoside phosphonate analog is phosphorylated by cellular kinases to the diphosphate and is incorporated into viral DNA by the viral DNA polymerase. With improved therapy of HIV infection, the incidence of CMV retinitis is low. Cidofovir and cyclic cidofovir inhibit the replication of both HHV-6A and HHV-6B with EC_{50} values of 3–9 μM [20,22•]. There are anecdotal reports suggesting that it may have some utility in the treatment of HHV-6 infections either alone or with other compounds and is plausible given this drug is active against all the human herpesviruses [33,42,44]. Drug resistance has been generated in the laboratory and maps to the HHV-6 DNA polymerase, namely the U38 gene [45].

Brincidofovir

The lipophilic derivative of cidofovir, brincidofovir or CMX001, is the hexadecyloxypropyl-cidofovir molecule [46]. It is a lipophilic prodrug of cidofovir that is highly active against many human DNA viruses, including HHV-6 and its mechanism of action is similar to that of CDV. It is among the most active molecules that have

been tested against this virus with EC_{50} values of 3 and 7 nM for HHV-6A and HHV-6B, respectively [47]. This molecule has been studied extensively in animal models against a variety of DNA viral infections, and has superior activity to cidofovir [48,49]; however, since there is no animal model of HHV-6 infection, no similar data are currently available. Furthermore, the drug was shown to suppress CMV disease in hematopoietic cell transplant in a phase 2 clinical trial, and a pivotal phase 3 clinical trial is underway in hematopoietic stem cell transplant recipients [50]. Since the betaherpesviruses CMV and HHV-6 both exhibit similar levels of susceptibility to brincidofovir, the drug might be expected to suppress disease from both viruses in this high risk population.

CMV423

CMV423 is a novel molecule with good activity against HHV-6 (EC_{50} approximately 50 nM) [51]. It inhibits a cellular protein tyrosine kinase that plays a critical role in HHV-6 viral replication [52]. Since the compound inhibits a cellular protein, the likelihood of advancement into clinical trials is unclear because of potential host cell toxicity.

Cyclopropavir and other methylenecyclopropane analogs

Cyclopropavir is a methylenecyclopropane analog that is a potent inhibitor of CMV and is also active *in vitro* against HHV-6 infection with EC_{50} values of 1 and 6 μM for HHV-6A and 6B [22•,53]. Its mechanism of action is similar to ganciclovir in that it is phosphorylated by the CMV UL97 kinase, but it also interferes with the normal function of the UL97 kinase that is critical to the replication of CMV [54,55,56•,57]. In HHV-6 the UL97 homolog, U69, phosphorylates the drug thus, its mechanism of action appears to parallel that of ganciclovir although with superior efficacy against HHV-6 [58••].

Recently, similar methylenecyclopropane analogs have been synthesized with ether and thioether substitutions at the 6 position of the purine and these analogs have superior activity to cyclopropavir [22•]. These are also phosphorylated directly by the U69 kinase [58••]. The mechanism of action of this series of compounds is more complex than that of cyclopropavir because of the modification of the guanine as well as the absence of a 3′ hydroxyl, which implies that it is likely an obligate chain terminator (Figure 1).

Benzimidazole derivatives

Several benzimidazole analogs have been reported to have antiviral activity against the human herpesviruses; maribavir is an L benzimidazole and is a potent inhibitor of CMV replication with submicromolar efficacy [59]. The molecule inhibits CMV UL97 kinase and is the most specific protein kinase inhibitor that has been identified to date [57]. Unfortunately, Phase III clinical trials for the prevention of CMV infection in hematopoietic stem cell transplant recipients failed to demonstrate efficacy and its further development is in doubt. This molecule also inhibits the U69 protein kinase in HHV-6, however its antiviral activity against HHV-6A and HHV-6B in cell culture is very limited as compared to CMV [60].

Another D benzimidazole analog, BDCRB (2-bromo-5, 6-dichloro-1-beta-D-ribofuranosylbenzimidazole), has a completely different mechanism of action and is the first described inhibitor of the CMV terminase [61]. While this molecule exhibits limited antiviral activity against HHV-6, the L analog of BDCRB is a potent inhibitor of HHV-6 with EC_{50} values of 2.8 and 9.7 μM for HHV-6A and HHV-6B, respectively [60]. It is unknown whether this compound targets the U69 kinase, terminase complex, or other essential function, but the distinct structure activity relationship of this series of compounds against HHV-6 is clearly different from that of CMV and thus this series of compounds holds promise.

Other molecules with activity against HHV-6 *in vitro*

Artesunate molecules are licensed and have efficacy against malaria and, to a much more limited extent, CMV infection. The precise mechanism of action against the DNA viruses is unknown and may not be specific. For CMV infection, the EC_{50} is approximately 5.8 μM [62,63]. Anecdotal reports have documented apparent efficacy in a very few cases but clinical trials will be required to assess the potential utility of this compound [64,65]. Efficacy has also been reported against HHV-6A with and EC_{50} value of 3.8 μM [66]. A single report described the use of the drug in the treatment of a child with HHV-6B myocarditis, yet it was unclear that the artesunate therapy was related to the recovery of the patient [67•].

Numerous other molecules have been reported to exhibit antiviral activity against HHV-6 in cell culture. 3-Deaza-HPMPA is active in the low μM range [20], however, because of toxicity it will not be advanced into clinical trials. Arylsulfone derivatives also have been reported to exhibit activity with CMV EC_{50} values at the low micromolar level and this series warrants further investigation [68]. Continued evaluation of new molecular entities will likely be required to identify potent new molecules with novel molecular targets.

Conclusions

At present, it is highly unlikely that any drug will be developed specifically for the treatment of HHV-6 infections. The availability of molecules that inhibit HHV-6 will in all probability result from spin-offs of those drugs synthesized to improved therapy of CMV infections of humans. More importantly, it underscores the need for

safe and effective broad spectrum antiviral drugs that can prevent disease in high risk populations not only from CMV and HHV-6, but also from other DNA viruses such as HHV-7, herpes simplex virus, Epstein-Barr virus, varicella-zoster virus, BK virus, and adenovirus. As long as the causative role of HHV-6 in diseases with significant impact or morbidity is not established, the development of specific therapeutics for this virus will remain a relatively low priority. The focus on antiviral agents with a broad spectrum antiviral activity that includes the roseoloviruses currently has the greatest potential to yield effective therapies for these infections.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Zerr DM *et al.*: **A population-based study of primary human herpesvirus 6 infection**. *N Engl J Med* 2005, **352**:768-776.
2. Dominguez G *et al.*: **Human herpesvirus 6B genome sequence: coding content and comparison with human herpesvirus 6A**. *J Virol* 1999, **73**:8040-8052.
3. Inoue N, Dambaugh TR, Pellett PE: **Molecular biology of human herpesviruses 6A and 6B**. *Infect Agents Dis* 1993, **2**:343-360.
4. Yamanishi K *et al.*: **Identification of human herpesvirus-6 as a causal agent for exanthem subitum**. *Lancet* 1988, **1**:1065-1067.
5. Steeper TA *et al.*: **The spectrum of clinical and laboratory findings resulting from human herpesvirus-6 (HHV-6) in patients with mononucleosis-like illnesses not resulting from Epstein-Barr virus or cytomegalovirus**. *Am J Clin Pathol* 1990, **93**:776-783.
6. Smith JM, McDonald RA: **Emerging viral infections in transplantation**. *Pediatr Transplant* 2006, **10(7)**:838-843.
7. Yoshikawa T: **Human herpesvirus-6 and -7 infections in transplantation**. *Pediatr Transplant* 2003, **7**:11-17.
8. Okuno T *et al.*: **Seroepidemiology of human herpesvirus 6 infection in normal children and adults**. *J Clin Microbiol* 1989, **27**:651-653.
9. Santoro F *et al.*: **CD46 is a cellular receptor for human herpesvirus 6**. *Cell* 1999, **99**:817-827.
10. Tang H *et al.*: **CD134 is a cellular receptor specific for human herpesvirus-6B entry**. *Proc Natl Acad Sci U S A* 2013, **110**:9096-9099.
11. He J *et al.*: **Infection of primary human fetal astrocytes by human herpesvirus 6**. *J Virol* 1996, **70**:1296-1300.
12. Isaacson E *et al.*: **Evidence of human herpesvirus 6 infection in 4 immunocompetent patients with encephalitis**. *Clin Infect Dis* 2005, **40**:890-893.
13. Soldan SS *et al.*: **Association of human herpes virus 6 (HHV-6) with multiple sclerosis: increased IgM response to HHV-6 early antigen and detection of serum HHV-6 DNA**. *Nat Med* 1997, **3**:1394-1397.
14. Boeckh M *et al.*: **Emerging viral infections after hematopoietic cell transplantation**. *Pediatr Transplant* 2005, **9(Suppl. 7)**:48-54.
15. Dulery R *et al.*: **Early human herpesvirus type 6 reactivation after allogeneic stem cell transplantation: a large-scale clinical study**. *Biol Blood Marrow Transplant* 2012, **18**:1080-1089.
16. Tokimasa S *et al.*: **Ganciclovir is effective for prophylaxis and treatment of human herpesvirus-6 in allogeneic stem cell transplantation**. *Bone Marrow Transplant* 2002, **29**:595-598.
17. Leong HN *et al.*: **The prevalence of chromosomally integrated human herpesvirus 6 genomes in the blood of UK blood donors**. *J Med Virol* 2007, **79**:45-51.
18. • Pellett PE *et al.*: **Chromosomally integrated human herpesvirus 6: questions and answers**. *Rev Med Virol* 2012, **22**:144-155.
This report provides consensus response to the unexpected finding that HHV-6 integrates into the telomeric regions of host DNA.
19. Black JB *et al.*: **Growth properties of human herpesvirus-6 strain Z29**. *J Virol Methods* 1989, **26**:133-145.
20. Reymen D *et al.*: **Antiviral activity of selected acyclic nucleoside analogues against human herpesvirus 6**. *Antivir Res* 1995, **28**:343-357.
21. Ablashi DV *et al.*: **Genomic polymorphism: growth properties, and immunologic variations in human herpesvirus-6 isolates**. *Virology* 1991, **184**:545-552.
22. • Prichard MN *et al.*: **Synthesis and antiviral activities of methylenecyclopropane analogs with 6-alkoxy and 6-alkylthio substitutions that exhibit broad-spectrum antiviral activity against human herpesviruses**. *Antimicrob Agents Chemother* 2013, **57**:3518-3527.
New analogs cyclopropavir with superior activity in HHV-6A and HHV-6B are described with a good description of analytic methods.
23. Manichanh C *et al.*: **Susceptibility of human herpesvirus 6 to antiviral compounds by flow cytometry analysis**. *Cytometry* 2000, **40**:135-140.
24. De Clercq E, Naesens L: **In search of effective anti-HHV-6 agents**. *J Clin Virol* 2006, **37(Suppl. 1)**:S82-S86.
25. Gerdemann U *et al.*: **Immunotherapeutic strategies to prevent and treat human herpesvirus 6 reactivation after allogeneic stem cell transplantation**. *Blood* 2013, **121**:207-218.
26. Yoshida M *et al.*: **Comparison of antiviral compounds against human herpesvirus 6 and 7**. *Antivir Res* 1998, **40**:73-84.
27. De Bolle L *et al.*: **Role of the human herpesvirus 6 u69-encoded kinase in the phosphorylation of ganciclovir**. *Mol Pharmacol* 2002, **62**:714-721.
28. De Bolle L *et al.*: **Human herpesvirus 6 infection arrests cord blood mononuclear cells in G(2) phase of the cell cycle**. *FEBS Lett* 2004, **560**:25-29.
29. De Bolle L *et al.*: **Human herpesvirus 6 DNA polymerase: enzymatic parameters, sensitivity to ganciclovir and determination of the role of the A961V mutation in HHV-6 ganciclovir resistance**. *Antivir Res* 2004, **64**:17-25.
30. Manichanh C *et al.*: **Selection of the same mutation in the U69 protein kinase gene of human herpesvirus-6 after prolonged exposure to ganciclovir in vitro and in vivo**. *J Gen Virol* 2001, **82**:2767-2776.
31. Ljungman P *et al.*: **Effectiveness of ganciclovir against human herpesvirus-6 excreted in saliva in stem cell transplant recipients**. *Bone Marrow Transplant* 2007, **39**:497-499.
32. Kogelnik AM *et al.*: **Use of valganciclovir in patients with elevated antibody titers against Human Herpesvirus-6 (HHV-6) and Epstein-Barr Virus (EBV) who were experiencing central nervous system dysfunction including long-standing fatigue**. *J Clin Virol* 2006, **37(Suppl. 1)**:S33-S38.
33. Mechai F *et al.*: **Human herpesvirus 6-associated retrobulbar optic neuritis in an HIV-infected patient: response to anti-herpesvirus therapy and long-term outcome**. *J Med Virol* 2007, **79**:931-934.
34. Cacheux W *et al.*: **HHV-6-related acute liver failure in two immunocompetent adults: favourable outcome after liver transplantation and/or ganciclovir therapy**. *J Intern Med* 2005, **258**:573-578.
35. Johnston RE *et al.*: **HHV-6-related secondary graft failure following allogeneic bone marrow transplantation**. *Br J Haematol* 1999, **105**:1041-1043.
36. Mookerjee BP, Vogelsang G: **Human herpes virus-6 encephalitis after bone marrow transplantation: successful treatment with ganciclovir**. *Bone Marrow Transplant* 1997, **20**:905-906.

37. Rieux C *et al.*: **Human herpesvirus-6 meningoencephalitis in a recipient of an unrelated allogeneic bone marrow transplantation**. *Transplantation* 1998, **65**:1408-1411.

38. •• Bounaadja L *et al.*: **Analysis of HHV-6 mutations in solid organ transplant recipients at the onset of cytomegalovirus disease and following treatment with intravenous ganciclovir or oral valganciclovir**. *J Clin Virol* 2013, **58**:279-282.
Resistance to ganciclovir and valganciclovir can occur during immune suppression and this report characterized the rate of resistance of HHV-6B as low during a course of therapy.

39. Isegawa Y *et al.*: **Human herpesvirus 6 ganciclovir-resistant strain with amino acid substitutions associated with the death of an allogeneic stem cell transplant recipient**. *J Clin Virol* 2009, **44**:15-19.

40. Shiraki K *et al.*: **Phosphonoacetic acid inhibits replication of human herpesvirus-6**. *Antivir Res* 1989, **12**:311-318.

41. Bethge W *et al.*: **Successful treatment of human herpesvirus-6 encephalitis after bone marrow transplantation**. *Bone Marrow Transplant* 1999, **24**:1245-1248.

42. Pohlmann C *et al.*: **Cidofovir and foscarnet for treatment of human herpesvirus 6 encephalitis in a neutropenic stem cell transplant recipient**. *Clin Infect Dis* 2007, **44**:e118-e120.

43. Bonnafous P *et al.*: **Different mutations in the HHV-6 DNA polymerase gene accounting for resistance to foscarnet**. *Antivir Ther* 2007, **12**:877-888.

44. Breddemann A *et al.*: **Case report: severe gastrointestinal inflammation and persistent HHV-6B infection in a paediatric cancer patient**. *Herpes* 2007, **14**:41-44.

45. Bonnafous P *et al.*: **Characterization of a cidofovir-resistant HHV-6 mutant obtained by in vitro selection**. *Antivir Res* 2008, **77**:237-240.

46. Hostetler KY: **Alkoxyalkyl prodrugs of acyclic nucleoside phosphonates enhance oral antiviral activity and reduce toxicity: current state of the art**. *Antivir Res* 2009, **82**:A84-A98.

47. Williams-Aziz SL *et al.*: **Comparative activities of lipid esters of cidofovir and cyclic cidofovir against replication of herpesviruses in vitro**. *Antimicrob Agents Chemother* 2005, **49**:3724-3733.

48. Bidanset DJ *et al.*: **Oral activity of ether lipid ester prodrugs of cidofovir against experimental human cytomegalovirus infection**. *J Infect Dis* 2004, **190**:499-503.

49. Quenelle DC *et al.*: **Oral treatment of cowpox and vaccinia virus infections in mice with ether lipid esters of cidofovir**. *Antimicrob Agents Chemother* 2004, **48**:404-412.

50. Marty FM *et al.*: **CMX001 to prevent cytomegalovirus disease in hematopoietic-cell transplantation**. *N Engl J Med* 2013, **369**:1227-1236.

51. Naesens L *et al.*: **Antiviral activity of diverse classes of broad-acting agents and natural compounds in HHV-6-infected lymphoblasts**. *J Clin Virol* 2006, **37(Suppl. 1)**:S69-S75.

52. De Bolle L *et al.*: **Potent: selective and cell-mediated inhibition of human herpesvirus 6 at an early stage of viral replication by the non-nucleoside compound CMV423**. *Biochem Pharmacol* 2004, **67**:325-336.

53. Kern ER *et al.*: **In vitro activity and mechanism of action of methylenecyclopropane analogs of nucleosides against herpesvirus replication**. *Antimicrob Agents Chemother* 2005, **49**:1039-1045.

54. James SH *et al.*: **Cyclopropavir inhibits the normal function of the human cytomegalovirus UL97 kinase**. *Antimicrob Agents Chemother* 2011, **55**:4682-4691.

55. Chou S, Bowlin TL: **Cytomegalovirus UL97 mutations affecting cyclopropavir and ganciclovir susceptibility**. *Antimicrob Agents Chemother* 2011, **55**:382-384.

56. • Gentry BG *et al.*: **Resistance of human cytomegalovirus to cyclopropavir maps to a base pair deletion in the open reading frame of UL97**. *Antimicrob Agents Chemother* 2013, **57**:4343-4348.
This report describes a new mutation in CMV that phosphorylates cyclopropavir, a promising new drug that is highly active against HHV-6 and CMV.

57. Prichard MN: **Function of human cytomegalovirus UL97 kinase in viral infection and its inhibition by maribavir**. *Rev Med Virol* 2009, **19**:215-229.

58. •• Komazin-Meredith G *et al.*: **Human herpesvirus 6 U69 kinase phosphorylates the methylenecyclopropane nucleosides cyclopropavir, MBX 2168, and MBX 1616 to their monophosphates**. *Antimicrob Agents Chemother* 2013, **57**:5760-5762.
The UL69 kinase form HHV-6 was shown for the first time to phosphorylate ganciclovir and cyclopropavir.

59. Biron KK *et al.*: **Potent and selective inhibition of human cytomegalovirus replication by 1263W94: a benzimidazole L-riboside with a unique mode of action**. *Antimicrob Agents Chemother* 2002, **46**:2365-2372.

60. Prichard MN *et al.*: **Benzimidazole analogs inhibit human herpesvirus 6**. *Antimicrob Agents Chemother* 2011, **55**:2442-2445.

61. Underwood MR *et al.*: **Inhibition of human cytomegalovirus DNA maturation by a benzimidazole ribonucleoside is mediated through the UL89 gene product**. *J Virol* 1998, **72**:717-725.

62. Efferth T *et al.*: **Antiviral activity of artesunate towards wild-type: recombinant, and ganciclovir-resistant human cytomegaloviruses**. *J Mol Med (Berl)* 2002, **80**:233-242.

63. Kaptein SJ *et al.*: **The anti-malaria drug artesunate inhibits replication of cytomegalovirus in vitro and in vivo**. *Antivir Res* 2006, **69**:60-69.

64. Shapira MY *et al.*: **Artesunate as a potent antiviral agent in a patient with late drug-resistant cytomegalovirus infection after hematopoietic stem cell transplantation**. *Clin Infect Dis* 2008, **46**:1455-1457.

65. Wolf DG *et al.*: **Human cytomegalovirus kinetics following institution of artesunate after hematopoietic stem cell transplantation**. *Antivir Res* 2011, **90**:183-186.

66. Milbradt J *et al.*: **Sensitivity of human herpesvirus 6 and other human herpesviruses to the broad-spectrum antiinfective drug artesunate**. *J Clin Virol* 2009, **46**:24-28.

67. • Hakacova N *et al.*: **First therapeutic use of Artesunate in treatment of human herpesvirus 6B myocarditis in a child**. *J Clin Virol* 2013, **57**:157-160.
For the first time artesunate was used for compassionate therapy for HHV-6 myocardits rather than ganciclovir, foscarget or cidofovir.

68. Naesens L *et al.*: **Antiviral properties of new arylsulfone derivatives with activity against human betaherpesviruses**. *Antivir Res* 2006, **72**:60-67.

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Current Opinion in Virology

Evidence linking HHV-6 with multiple sclerosis: an update

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Following reports of elevated antiviral antibodies in MS patient sera and viral DNA detection in MS plaques nearly two decades ago, the neurovirology community has actively explored how herpesviruses such as HHV-6 might be involved in MS disease pathogenesis. Though findings across the field are non-uniform, an emerging consensus of viral correlates with disease course and evidence of HHV-6-specific immune responses in the CNS provide compelling evidence for a role, direct or indirect, of this virus in MS. Ultimately, the only way to demonstrate the involvement, or lack thereof, of HHV-6 or other herpesviruses in this disease is through a controlled clinical trial of an efficacious antiviral drug.

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Introduction: pathogens in multiple sclerosis

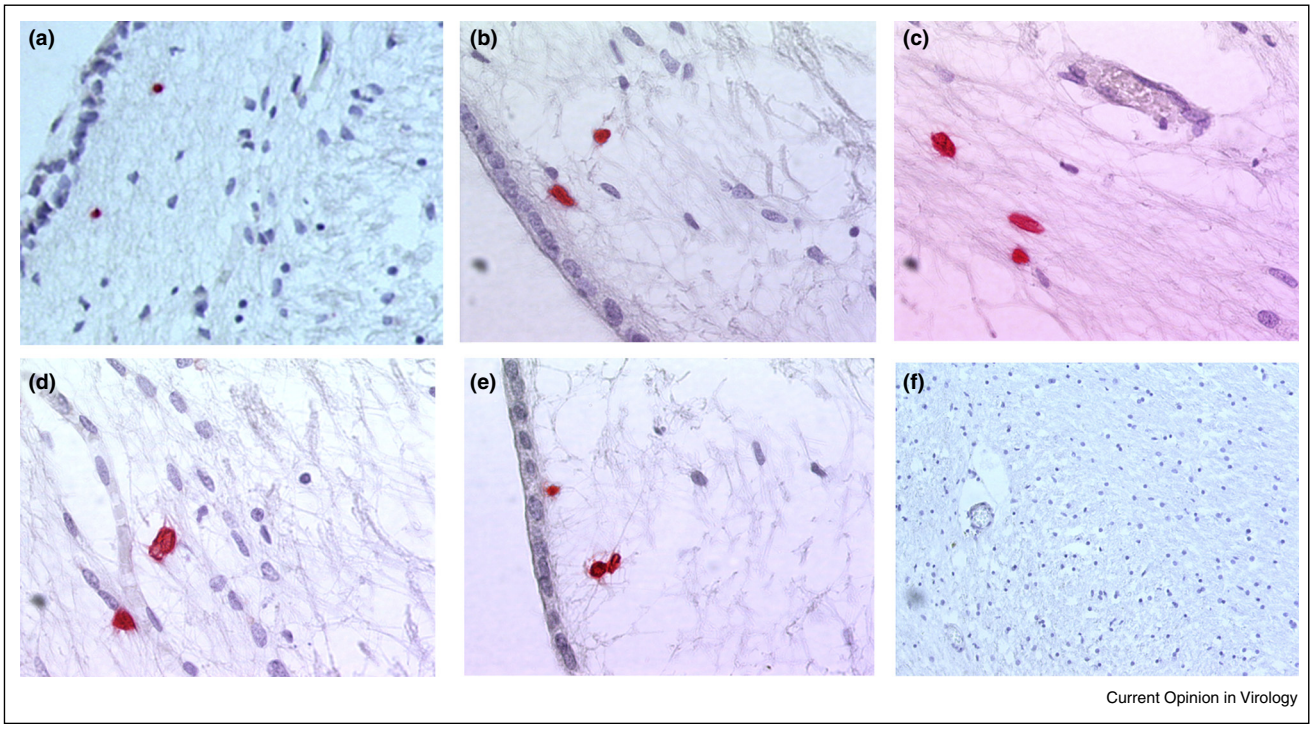
Multiple sclerosis (MS), a neurodegenerative, inflammatory demyelinating disease of the central nervous system (CNS), is idiopathic, despite its description over 150 years ago [1]. For the past two decades, following reports of elevated anti-human herpesvirus 6 (HHV-6) antibodies in MS patient sera [2,3] and HHV-6 viral DNA detection in MS plaques [4], the neurovirology community has actively explored if and how this virus is involved in MS disease pathogenesis.

The discussion of any pathogen implicated in MS should be contextualized by the long history of infectious agents in this disease. Proponents of an infectious etiology of MS can be traced back to the mid 19th century, when descriptions of the disease were beginning to coalesce [1]. The idea of an infectious etiology resurged in the 1930s with the observation that, by histopathology, the perivenous demyelination of MS and post-infectious encephalomyelitis were indistinguishable. From this time forward, there were many reports of agents detected in MS patient spinal fluid including spirochetes and *Toxoplasma gondii* [1]. There were also reports of agents recovered from laboratory animals following immunization with tissue from MS patients. These agents have been largely dismissed due to confirmed contamination or irreproducibility, but the list once included rabies, a Scrapie agent, measles and chimpanzee cytomegalovirus, to name a few. Interestingly, viruses have dominated the list of suspected agents; there have been few bacteria or parasites by comparison [5••]. However, despite the subsequent isolation of the specific viruses responsible for the demyelinating diseases subacute sclerosing panencephalitis (SSPE: measles virus) and progressive multifocal leukoencephalopathy (PML: JC virus), the focus of the MS field has largely transitioned away from a single, unidentified agent (though some hold this view [6]) towards ubiquitous agents, particularly herpesviruses [5••]. While there are numerous reports for other herpesviruses in MS, notably the sero-epidemiological data for human herpesvirus 4 (Epstein–Barr virus (EBV)) reviewed in [7,8], this current review will focus solely on HHV-6.

Traces of HHV-6 in the CNS: virus detection and virus-specific immune responses

Early studies reporting HHV-6 viral DNA in the brains [9,10] and CSF [11] of MS patients and controls supported that HHV-6 possessed strong neurotropism that was associated with a CNS reservoir [9]. This was supported by concomitant studies reporting higher levels of HHV-6 expression in MS brains compared to control brains [12], and greater levels of viral DNA [13,14] and viral mRNA [12] specifically in the demyelinated plaques. An example of HHV-6 expression, as detected by immunohistochemistry (IHC), in a periventricular MS lesion is shown in Figure 1. HHV-6 positivity (red) is evident in the lesion (a–e), but notably absent in non-lesional areas and normal appearing white matter (f). The observations of viral mRNA [12] and protein expression [4] specifically in oligodendrocytes proved central to the hypothesis that HHV-6 may be a driver of MS pathogenesis. Collectively, these studies demonstrated that while HHV-6 may be a commensal of normal brain, its replication and activity is enriched in the context of MS pathology. This is highlighted in Table 1, which summarizes the

Figure 1



HHV-6 expression is detectable by immunohistochemistry in a periventricular MS lesion **(a–e)**, but not in the normal appearing white matter **(f)**. Red: HHV-6 gp116. MS lesions were obtained from a subset of patient material previously reported [14].

pathologic, inflammatory and virologic findings of 20 lesions from a subset of MS lesions previously reported [14]. HHV-6 expression was greater in the acute relative to chronic lesions, associating viral expression with earlier stages of MS lesion formation. This appears specific for HHV-6 since IHC for three other herpesviruses were uniformly negative (Table 1).

Compelling evidence that HHV-6 may be a key component in MS pathology stems from the observation that in approximately 20% of patients, a subset of oligoclonal bands (OCB) demonstrates HHV-6 specificity [15,16]. A 2014 publication by Pietläinen-Nicklén and colleagues analyzed patients with demyelinating disease (mostly MS) and HHV-6-reactive CSF OCB, and determined that patients with HHV-6 OCB appear to form a separate group, which was significantly younger, with greater IgG OCB relative to patients without HHV-6 OCB [17]. OCB, representing intrathecally produced immunoglobulins, are a hallmark of MS but are not specific for the disease. In fact, OCB are common among CNS disorders with an infectious component, and when the inciting agent is known, OCB are often specific to that agent (for example measles virus in SSPE). For this reason, the identification of HHV-6-specific bands in a subset of MS patients has strengthened the idea that HHV-6 from within the CNS is involved in the disease (Figure 2) [18]. Furthermore, the hypothesis of an antigen-driven immune response in MS is supported by data of clonally expanded B cells in MS brains, similar to SSPE brains [19]. A recent study observed interesting correlates between the presence of herpesvirus-specific OCB (HHV-6 and EBV) and several clinical parameters [20]. Virtanen and colleagues reported that herpesvirus-specific CSF OCB inversely correlated with the detection of CSF viral DNA, and that MS patients with CSF viral DNA had significantly more contrast enhancing lesions compared to those without detectable CSF viral DNA. These data suggest that anti-viral antibodies may be necessary for the maintenance of viral latency, as the reduction in such antibodies corresponded to both detectable CSF virus and MRI activity indicative of an active inflammatory process [20].

While OCB reflect CNS B cell reactivity toward HHV-6, less is known about CNS T cell reactivity toward HHV-6. A recent study by Wuest and colleagues reported significant enrichment of HHV-6 specific CD4 T cell responses in CSF compared to peripheral blood of MS patients (progressive and relapsing-remitting subtypes), suggesting that HHV-6-expanded T cells in the CNS may contribute to disease activity [21].

Table 1

MS lesion activity and viral infection

Lesion	Classification	Lesion pathology				Inflammation				Herpesviral expression			
		Axonal damage (NFTP)	Astrocytosis (GFAP)	Myelin loss (LFB)	Oligo loss (S100)	CD4+ T cells	CD8+ T cells	CD20+ B cells	CD68+ MΦ	HSV-1	CMV	EBV	HHV-6
1	Acute	Intact	Reactive	Minor	Normal	++	++	–	++	–	–	–	+
2	Acute	Intact	Reactive	Major	Normal	+	++	–	+++	–	–	–	+++
3	Chronic active	Major	Reactive	Major	Minor	++	+	–	+	–	–	–	+
4	Chronic	Major	Normal	Major	Major	–	+	–	+	–	–	–	+
5	Acute	Minor	Reactive	Minor	Normal	++	++	–	+++	–	–	–	++
6	Acute	Intact	Reactive	Minor	Normal	++	++	–	++	–	–	–	++
7	Chronic active	Intact	Reactive	Major	Normal	–	+	–	+++	–	–	–	+
8	Acute	Intact	Reactive	Minor	Normal	–	+	–	+++	–	–	–	++
9	Acute	Minor	Reactive	Minor	Normal	+	+	–	++	–	–	–	+
10	Chronic	Major	Normal	Major	Major	–	–	–	–	–	–	–	–
11	Acute	Intact	Reactive	Minor	Normal	+	+	–	++	–	–	–	++
12	Acute	Minor	Reactive	Minor	Normal	+	+	–	++	–	–	–	+
13	Chronic active	Major	Normal	Major	Minor	+	+	–	+	–	–	–	+
14	Chronic active	Intact	Reactive	Major	Normal	+	+	+	++	–	–	–	+
15	Chronic	Minor	Reactive	Major	Minor	+	+	–	++	–	–	–	–
16	Acute	Minor	Reactive	Major	Normal	–	+	+	+++	–	–	–	+
17	Chronic active	Minor	Reactive	Major	Normal	+	+	–	++	–	–	–	–
18	Chronic active	Minor	Reactive	Major	Minor	+	++	+	++	–	–	–	+
19	Chronic active	Major	Reactive	Major	Major	++	++	++	+	–	–	–	–
20	Shadow	Minor	Normal	Minor	Normal	–	–	–	+	–	–	–	++

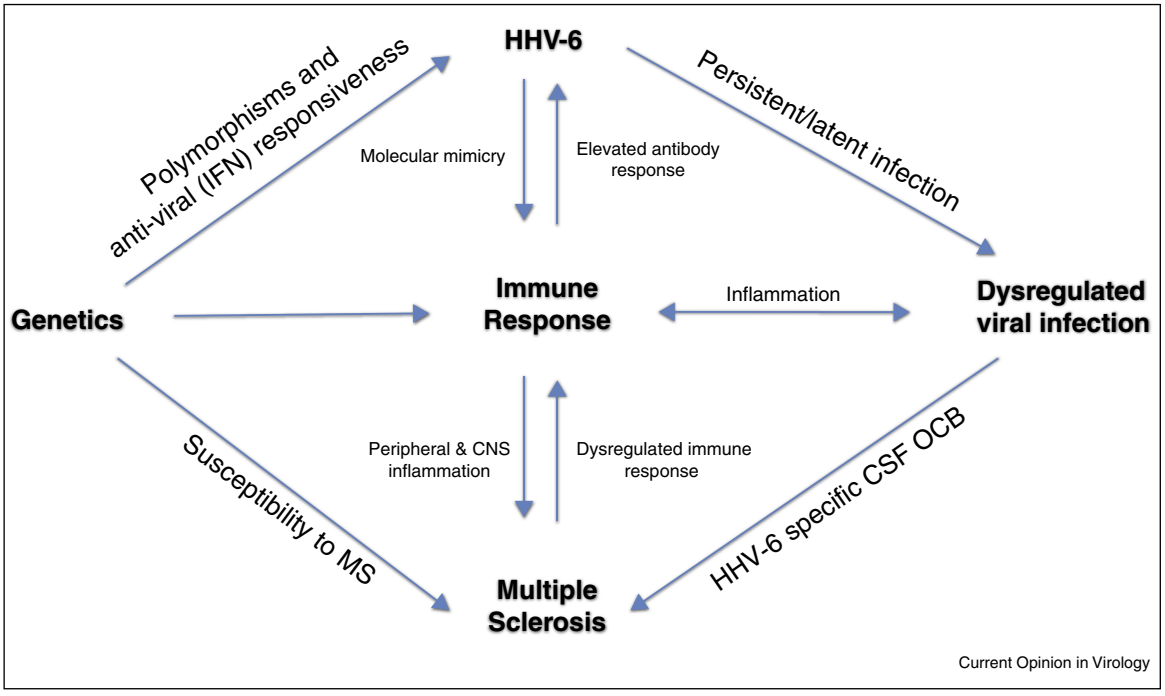
Traces of HHV-6 in the periphery: virus detection and virus-specific immune responses

It is not solely studies of the CNS that have established an association between HHV-6 and MS; early observations of HHV-6 in the periphery of MS patients linked the detection of, or an immune response to, the virus with clinically active disease [22,23]. Recent studies with MS cohorts in different geographical areas have largely confirmed these previously reported observations. Two recent studies found greater levels of HHV-6 IgM and IgG in MS cohorts compared to controls, one in an Iranian population [24] and one in a Tunisian population [25•]. A separate study of another Iranian MS cohort detected a higher frequency of viral DNA in the serum of patients, along with a relative increase in viral load during disease exacerbation [26]. Such observations of increased antibody responses and elevated viral loads in the serum, especially during disease exacerbation, confirm earlier observations of HHV-6 in MS and appear to be valid across geographically varied populations.

Many serologic and DNA studies published in the past several years have stratified MS patients into the clinical phases of relapse or remission, and provide mounting evidence for a role — direct or indirect — of HHV-6 in the switch from remission to relapse. A 2012 study of a Tasmanian cohort found HHV-6 IgG titer to be a significant predictor of relapse risk [27]. This was echoed in a 2014 study of a Spanish MS cohort, which reported that a decrease in HHV-6 antibody titers correlated with fewer relapses and less disease progression [28]. Interestingly, the authors noted that IgG titers reached their highest value two weeks, and IgM titers one month, before relapse [28]. A 2011 study of a Latvian MS cohort reported HHV-6 DNA in the plasma of a majority of RRMS and SPMS patients during relapse, which was confirmed by enhancing MRI lesions, and correlated with higher serum concentrations of the inflammatory cytokines IL-12 and TNF-alpha relative to periods of remission [29•]. These data agree with earlier studies of serum HHV-6 detection during relapse and add the observation of cytokine correlates, complementing a recent study suggesting that TNF-alpha may be predictive of HHV-6 reactivation [30]. However, if HHV-6 is involved in relapses, the nature of its involvement remains unknown. Does the virus have an active role in initiating or potentiating the inflammation associated with relapse, or is it a marker of disease activity, activated from latency as a result of the surrounding inflammation?

Other serological studies have focused on the immune response to a specific portion of the virus, an approach that may provide functional insights into the role of HHV-6 in disease. A 2013 study examined antibodies to a latency-promoting protein, U94/REP, and found elevated IgG levels in Tunisian MS patients compared to controls; for eight patients with samples collected during relapsing

Figure 2



A complex interplay between genetics, immune response and viral infections (such as HHV-6) influences the development of MS. Genetics have been implicated in the susceptibility to the disease, as well as in the response to antiviral therapy. Under certain inflammatory conditions, potentially in genetically susceptible individuals, the latency and persistence of herpesviruses may result in a dysregulated infection. Anti-viral immune responses in the periphery and CNS of MS patients suggest that a dysregulated viral infection is a key component of the disease.
Adapted from Owens, Bennet. 2012 Mult Scler.

and remitting phases, significantly higher titers were detected during the relapsing phases [31]. The finding of an elevated U94 IgG response in MS patients versus controls agrees with previous findings in an Italian cohort [32], and adds the observation of higher titers during relapse versus remission. Elevated antibodies against a latency-promoting protein may be one mechanism leading to the increased viral levels observed across many MS cohorts. Another approach to investigating the immune response against a specific viral protein is identification of the antigenic target of anti-viral antibodies. In a recently published study, Alenda and colleagues purified IgG from the CSF of RRMS and PPMS patients, then incubated the IgG with HHV-6 and characterized peptides of the bound antigens. They reported that the peptides matched the major capsid protein of HHV-6A, a structural protein needed to assemble the viral capsid [33•]. This approach provides a framework for exploring the antigenic targets of HHV-6 antibodies, and whether there are differences between the periphery and CSF, MS patients and controls or MS patients in different stages of the disease.

HHV-6 status post-interferon treatment: examining the influence of polymorphisms

A long-standing argument in support of a viral etiology of MS is the effectiveness of interferon beta, a potent antiviral [34]. Several studies published in the past few years have formally examined the relationship between interferon treatment and HHV-6 status in MS patients. In a 2011 publication, Garcia-Montojo and colleagues observed that patients with HHV-6 viral DNA in whole blood and serum exhibited a higher risk of MS relapse and comprised a lower proportion of IFN-beta-1b responders [35]. These data agree with the many studies that detect an increase in serum viral DNA during relapse compared to remission, and add the observation of an inverse correlation with IFN-beta responsiveness.

Several studies have adopted a gene-environment interactions approach to the study of HHV-6 and interferon therapy, correlating polymorphisms with HHV-6 status and therapy responsiveness. For instance, Vandenbroeck and colleagues reported elevated odds ratios for specific polymorphisms of the transcription factor IRF5 (interferon regulatory factor 5) and HHV-6 infection and interferon responsiveness [36]. In a separate study, Garcia-Montojo and colleagues studied polymorphisms in MHC2TA, which encodes a transcriptional coactivator of MHC class II genes, and reported significant differences in genotype frequency between MS patients with and without detectable serum HHV-6 [37]. In a follow up study, they observed that a significantly higher proportion

of MS patients with higher MHC2TA mRNA levels and without detectable serum HHV-6 were clinical responders to interferon beta therapies compared to patients with decreased MHC2TA mRNA levels and detectable serum HHV-6. The authors concluded that MHC2TA mRNA levels might be decreased by the active replication of HHV-6 [38•]. Interestingly, human cytomegalovirus (HCMV), a beta herpesvirus like HHV-6, has been reported to decrease MHC2TA mRNA levels, resulting in the suppression of MHC class II expression [39]. While this study found no correlation between polymorphisms and the development of interferon-neutralizing antibodies [38•], future studies should examine polymorphisms that correlate with interferon-neutralizing antibodies and HHV-6 viral DNA.

Potential mechanism of HHV-6 involvement in MS: molecular mimicry with myelin

Associations of viruses with human demyelinating disease and virally induced animal models of demyelination provide compelling, though indirect, evidence of a viral etiology of MS [19]. Additionally, studies of mechanisms of demyelination and oligodendrocyte injury have reinforced the idea that viruses can lead to MS or MS-like pathology [5••]. One such mechanism is molecular mimicry, whereby sequence homology between a pathogen and a self-molecule leads to the generation of an immune response that is cross-reactive between both the pathogen and self. There is a stretch of identical amino acids between HHV-6 U24 (an integral membrane protein [40]) and human myelin basic protein (MBP), which has bolstered the idea that molecular mimicry may be at play in the relationship between HHV-6 and MS. In 2003, Tejada-Simon and colleagues reported that MS patients, compared to healthy controls, exhibited a much higher frequency of T cells that were reactive to both $(\text{HHV-6 U24})_{1–13}$ and $(\text{MBP})_{93–105}$ [41]. These observations were recently confirmed in a cohort of Chinese MS patients, in a 2012 study by Cheng and colleagues [42].

While positive findings continue to provide an impetus to study the role of HHV-6 in MS, much about the mechanisms remain unknown. Are elevated levels of HHV-6 in MS a hallmark of an aberrant immune response or a reflection of the failure of the immune response to contain infection (Figure 2)? As inflammation can induce reactivation in T cells trafficking through the CNS [19], to what extent is the virus causal or simply a reactivated byproduct of vast peripheral and CNS inflammation?

Controversy: findings and suggestions

Despite a publication bias toward positive results, not all published reports of HHV-6 in MS are positive; several recent studies have found a non-significant or low incidence of HHV-6 in their respective MS populations. A 2014 study of South African MS patients and controls reported no difference between HHV-6 viral DNA detection in whole blood between MS patients and controls [43]. Another study of Swedish patients reported a low incidence of HHV-6 in the plasma and CSF of possible MS patients compared to controls. These investigators also detected a low incidence of HHV-6 in the serum samples of IFN-beta treated MS patients, without any difference between patients with or without neutralizing antibodies [44]. Another study of a Tasmanian MS cohort prospectively analyzed levels of HHV-6 IgM as a marker of viral reactivation; the authors detected HHV-6 IgM in only 1/198 patients, and concluded that HHV-6 reactivation does not drive relapse or disability in this MS population [45].

Many factors can account for discordant results, including differences in patient and control populations, technical differences and the timing of sample acquisition, to name a few. In a multitude of positive studies, HHV-6 appears in only a subset of MS patients and yet, the findings are often interpreted broadly. Investigators of both positive and negative studies should carefully parse out characteristics of the patient and control populations in question, in an effort to foster hypothesis generation and present more nuanced conclusions than HHV-6 is or is not involved in MS.

Future directions

Ultimately, a controlled clinical trial of an efficacious [CNS penetrable] anti-HHV-6 drug in MS may be the only way to ascertain the involvement of this agent in MS (it is important to consider that a positive outcome demonstrating robust clinical efficacy would be persuasive, while a negative outcome would only add controversy to the field). However, additional basic research on the biology of HHV-6 — especially differences between the two viruses that comprise this group [46•] — is required for the discovery or development of such an anti-viral. For example, several studies have reported more HHV-6A relative to HHV-6B in MS patient material [38•,47,48]. Understanding the properties of each virus and knowing to what extent one or both are involved in MS is crucial to furthering this field, and all publications should diligently distinguish HHV-6A from HHV-6B viral DNA sequences. Serological differentiation between these two viruses is an active area of research [49] and once validated, will provide great insight into the relative antibody reactivity to each virus, and importantly, the time of acquisition of HHV-6A. The acquisition time of one or both viruses may be a factor in MS development, as has been proposed for EBV [50].

Sequencing additional HHV-6 genomes may also lead to a more thorough understanding of each virus. A 2013 study examined the oral shedding of EBV from pediatric MS patients and controls, and reported that changes in the predominant EBV variants were higher

in MS patients, suggesting a lack of immunologic control of this virus [51]. Perhaps there are different frequencies of HHV-6 variants between MS patients and controls? Or perhaps there are HHV-6 variants that differ between sites, for example the periphery and CNS? Studies of JC virus have identified sequences that lend to its classification as non-virulent (found in non-PML patients) or virulent (found in the brain and CSF of PML patients) [52]. As HHV-6 is at once ubiquitous and implicated in a non-ubiquitous pathology, perhaps there are genetic variants that are analogously associated with MS.

In conclusion, there is sufficient and compelling evidence that HHV-6 may be involved, albeit to an unknown extent, in the disease pathogenesis of a subset of MS cases. To elucidate the possible mechanisms of HHV-6A and/or HHV-6B involvement in this disease, or the involvement of other herpesviruses, future studies are encouraged to ask focused questions, using material from well-characterized patient populations and well-matched control populations.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Kesselring J: **History of multiple sclerosis**. In *Multiple Sclerosis: A Comprehensive Text.* Edited by Raine CS, McFarland HF, Hohlfeld R. Saunders: Elsevier; 2008:474.
2. Sola P *et al.*: **Human herpesvirus 6 and multiple sclerosis: survey of anti-HHV-6 antibodies by immunofluorescence analysis and of viral sequences by polymerase chain reaction**. *J Neurol Neurosurg Psychiatry* 1993, **56**:917-919.
3. Wilborn F *et al.*: **A potential role for human herpesvirus type 6 in nervous system disease**. *J Neuroimmunol* 1994, **49**:213-214.
4. Challoner PB *et al.*: **Plaque-associated expression of human herpesvirus 6 in multiple sclerosis**. *Proc Natl Acad Sci U S A* 1995, **92**:7440-7444.
5. •• Venkatesan A, Johnson RT: **Infections and multiple sclerosis**. In *Handbook of Clinical Neurology*. Edited by Goodin DS. Elsevier B.V.; 2014.

This chapter provides an exhaustive overview of infections in multiple sclerosis, highlighting animal models of viral demyelination, mechanisms of virally-induced demyelination and the ways by which infectious agents, of both historic and current interest, were implicated in multiple sclerosis.

6. Lipton HL *et al.*: **A specific viral cause of multiple sclerosis: one virus, one disease**. *Ann Neurol* 2007, **61**:514-523.
7. Lucas RM *et al.*: **Epstein–Barr virus and multiple sclerosis**. *J Neurol Neurosurg Psychiatry* 2011, **82**:1142-1148.
8. Pakpoor J, Giovannoni G, Ramagopalan SV: **Epstein–Barr virus and multiple sclerosis: association or causation?** *Expert Rev Neurother* 2013, **13**:287-297.
9. Merelli E *et al.*: **Human herpes virus 6 and human herpes virus 8 DNA sequences in brains of multiple sclerosis patients, normal adults and children**. *J Neurol* 1997, **244**:450-454.
10. Gordon L, McQuaid S, Cosby SL: **Detection of herpes simplex virus (types 1 and 2) and human herpesvirus 6 DNA in human brain tissue by polymerase chain reaction**. *Clin Diagn Virol* 1996, **6**:33-40.
11. Liedtke W *et al.*: **Human herpesvirus 6 polymerase chain reaction findings in human immunodeficiency virus associated neurological disease and multiple sclerosis**. *J Neurovirol* 1995, **1**:253-258.
12. Opsahl ML, Kennedy PG: **Early and late HHV-6 gene transcripts in multiple sclerosis lesions and normal appearing white matter**. *Brain* 2005, **128**:516-527.
13. Sanders VJ *et al.*: **Detection of herpesviridae in postmortem multiple sclerosis brain tissue and controls by polymerase chain reaction**. *J Neurovirol* 1996, **2**:249-258.
14. Cermelli C *et al.*: **High frequency of human herpesvirus 6 DNA in multiple sclerosis plaques isolated by laser microdissection**. *J Infect Dis* 2003, **187**:1377-1387.
15. Derfuss T, Hohlfeld R, Meinl E: **Intrathecal antibody (IgG) production against human herpesvirus type 6 occurs in about 20% of multiple sclerosis patients and might be linked to a polyspecific B-cell response**. *J Neurol* 2005, **252**:968-971.
16. Virtanen JO *et al.*: **Intrathecal human herpesvirus 6 antibodies in multiple sclerosis and other demyelinating diseases presenting as oligoclonal bands in cerebrospinal fluid**. *J Neuroimmunol* 2011, **237**:93-97.
17. Pietilainen-Nicklen J *et al.*: **HHV-6-positivity in diseases with demyelination**. *J Clin Virol* 2014.
18. Gilden DH: **Infectious causes of multiple sclerosis**. *Lancet Neurol* 2005, **4**:195-202.
19. Bennet JL, Yu X, Gilden DH, Burgoon MP, Owens GP: **Infectious agents and multiple sclerosis**. In *Multiple Sclerosis: A Comprehensive Text.* Edited by Raine CS, McFarland HF, Hohlfeld R. Saunders: Elsevier; 2008:480.
20. Virtanen JO *et al.*: **Oligoclonal bands in multiple sclerosis reactive against two herpesviruses and association with magnetic resonance imaging findings**. *Mult Scler* 2014, **20**:27-34.
21. Wuest SC *et al.*: **A complex role of herpes viruses in the disease process of multiple sclerosis**. *PLOS ONE* 2014, **9**:pe105434.
22. Soldan SS *et al.*: **Association of human herpes virus 6 (HHV-6) with multiple sclerosis: increased IgM response to HHV-6 early antigen and detection of serum HHV-6 DNA**. *Nat Med* 1997, **3**:1394-1397 [see comments].
23. Berti R *et al.*: **Increased detection of serum HHV-6 DNA sequences during multiple sclerosis (MS) exacerbations and correlation with parameters of MS disease progression**. *J Neurovirol* 2002, **8**:250-256.
24. Khaki M *et al.*: **Evaluation of viral antibodies in Iranian multiple sclerosis patients**. *Neurosciences (Riyadh)* 2011, **16**:224-228.
25. • Ramroodi N *et al.*: **Monitoring of active human herpes virus 6 infection in Iranian patients with different subtypes of multiple sclerosis**. *J Pathog* 2013, **2013**:194932.

This comprehensive study of Iranian MS patients and controls examines IgM, IgG and viral sequences in PBMC, saliva and serum, and extends previous observations of a systemically reactivated HHV-6 infection in MS patients compared to controls.

26. Behzad-Behbahani A *et al.*: **Human herpesvirus-6 viral load and antibody titer in serum samples of patients with multiple sclerosis**. *J Microbiol Immunol Infect* 2011, **44**:247-251.
27. Simpson S Jr *et al.*: **Anti-HHV-6 IgG titer significantly predicts subsequent relapse risk in multiple sclerosis**. *Mult Scler* 2012, **18**:799-806.
28. Ortega-Madueno I *et al.*: **Anti-human herpesvirus 6A/B IgG correlates with relapses and progression in multiple sclerosis**. *PLOS ONE* 2014, **9**:pe104836.
29. • Nora-Krukle Z *et al.*: **Human herpesvirus 6 and 7 reactivation and disease activity in multiple sclerosis**. *Medicina (Kaunas)* 2011, **47**:527-531.

This study correlates serum concentrations of IL-12 and TNF-alpha with active HHV-6 infection and relapse in RRMS and SPMS patients.

30. Uno H *et al.*: **TNF-alpha as a useful predictor of human herpesvirus-6 reactivation and indicator of the disease process in drug-induced hypersensitivity syndrome (DIHS)/ drug reaction with eosinophilia and systemic symptoms (DRESS)**. *J Dermatol Sci* 2014, **74**:177-179.

31. Ben-Fredj N *et al.*: **Prevalence of human herpesvirus U94/REP antibodies and DNA in Tunisian multiple sclerosis patients**. *J Neurovirol* 2013, **19**:42-47.

32. Caselli E *et al.*: **Detection of antibodies directed against human herpesvirus 6 U94/REP in sera of patients affected by multiple sclerosis**. *J Clin Microbiol* 2002, **40**:4131-4137.

33. • Alenda R *et al.*: **Identification of the major HHV-6 antigen recognized by cerebrospinal fluid IgG in multiple sclerosis**. *Eur J Neurol* 2014, **21**:1096-1101.
This study developed a method to purify and identify the HHV-6 antigens that are recognized by the IgG in MS patient CSF.

34. Dhib-Jalbut S, Marks S: **Interferon-beta mechanisms of action in multiple sclerosis**. *Neurology* 2010, **74**:S17-S24.

35. Garcia-Montojo M *et al.*: **Human herpesvirus 6 and effectiveness of interferon beta1b in multiple sclerosis patients**. *Eur J Neurol* 2011, **18**:1027-1035.

36. Vandenbroeck K *et al.*: **Validation of IRF5 as multiple sclerosis risk gene: putative role in interferon beta therapy and human herpes virus-6 infection**. *Genes Immun* 2011, **12**:40-45.

37. Garcia-Montojo M *et al.*: **Herpesvirus active replication in multiple sclerosis: a genetic control?** *J Neurol Sci* 2011, **311**:98-102.

38. • Dominguez-Mozo MI *et al.*: **MHC2TA mRNA levels and human herpesvirus 6 in multiple sclerosis patients treated with interferon beta along two-year follow-up**. *BMC Neurol* 2012, **12**:107.
This study found that the absence of HHV-6 viral DNA in serum and the increase of MHC2TA expression may serve as biomarkers predictive of response to interferon therapy.

39. Lee AW *et al.*: **Human cytomegalovirus decreases constitutive transcription of MHC class II genes in mature Langerhans cells by reducing CIITA transcript levels**. *Mol Immunol* 2011, **48**:1160-1167.

40. Sullivan BM, Coscoy L: **The U24 protein from human herpesvirus 6 and 7 affects endocytic recycling**. *J Virol* 2010, **84**:1265-1275.

41. Tejada-Simon MV *et al.*: **Cross-reactivity with myelin basic protein and human herpesvirus-6 in multiple sclerosis**. *Ann Neurol* 2003, **53**:189-197.

42. Cheng W *et al.*: **Cross-reactivity of autoreactive T cells with MBP and viral antigens in patients with MS**. *Front Biosci (Landmark Ed)* 2012, **17**:1648-1658.

43. Hon GM, Erasmus RT, Matsha T: **Low prevalence of human herpesvirus-6 and varicella zoster virus in blood of multiple sclerosis patients, irrespective of inflammatory status or disease progression**. *J Clin Neurosci* 2014, **21**:1437-1440.

44. Gustafsson R *et al.*: **Incidence of human herpesvirus 6 in clinical samples from Swedish patients with demyelinating diseases**. *J Microbiol Immunol Infect* 2013.

45. Simpson S Jr *et al.*: **EBV & HHV6 reactivation is infrequent and not associated with MS clinical course**. *Acta Neurol Scand* 2014.

46. • Ablashi D *et al.*: **Classification of HHV-6A and HHV-6B as distinct viruses**. *Arch Virol* 2014, **159**:863-870.
This review highlights the distinctions between HHV-6A and HHV-6B (biological, immunological, epidemiological) that support their recent reclassifcation from viral variants to distinct viral species.

47. Leibovitch EC *et al.*: **Coinfection of human herpesviruses 6A (HHV-6A) and HHV-6B as demonstrated by novel digital droplet PCR assay**. *PLOS ONE* 2014, **9**:pe92328.

48. Akhyani N *et al.*: **Tissue distribution and variant characterization of human herpesvirus (HHV)-6: increased prevalence of HHV-6A in patients with multiple sclerosis**. *J Infect Dis* 2000, **182**:1321-1325.

49. Higashimoto Y *et al.*: **Development of a human herpesvirus 6 species-specific immunoblotting assay**. *J Clin Microbiol* 2012, **50**:1245-1251.

50. Haahr S *et al.*: **A role of late Epstein–Barr virus infection in multiple sclerosis**. *Acta Neurol Scand* 2004, **109**:270-275.

51. Yea C *et al.*: **Epstein–Barr virus in oral shedding of children with multiple sclerosis**. *Neurology* 2013, **81**:1392-1399.

52. Ryschkewitsch CF, Jensen PN, Major EO: **Multiplex qPCR assay for ultra sensitive detection of JCV DNA with simultaneous identification of genotypes that discriminates non-virulent from virulent variants**. *J Clin Virol* 2013, **57**:243-248.

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Current Opinion in Virology

Chromosomally integrated HHV-6: impact on virus, cell and organismal biology

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HHV-6 integrates its genome into telomeres of human chromosomes. Integration can occur in somatic cells or gametes, the latter leading to individuals harboring the HHV-6 genome in every cell. This condition is transmitted to descendants and referred to as inherited chromosomally integrated human herpesvirus 6 (iciHHV-6). Although integration can occur in different chromosomes, it invariably takes place in the telomere region. This integration mechanism represents a way to maintain the virus genome during latency, which is so far unique amongst human herpesviruses. Recent work provides evidence that the integrated HHV-6 genome can be mobilized from the host chromosome, resulting in the onset of disease. Details on required structural determinants, putative integration mechanisms and biological and medical consequences of iciHHV-6 are discussed.

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Introduction

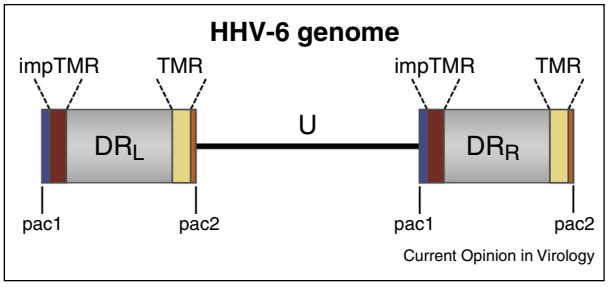
The genomes of human herpesvirus 6A (HHV-6A) and HHV-6B consist of a single unique segment (U) (~145 kbp) flanked by identical direct repeats (DR) (~9kbp) [1–4]. The DRs are flanked by pac1 and pac2 sequences that are involved in the cleavage and the packaging of the HHV-6 genome (Figure 1) [5,6]. Adjacent to the pac2 sequences is an array of telomeric repeats (TMR) that are identical to the human telomere sequences (TTAGGG). In proximity to pac1 is a second telomere array, consisting of TMR that are disrupted by other repetitive sequences, termed imperfect TMR (impTMR) [3,6]. Intriguingly, TMR are found in several lymphotropic herpesviruses belonging to the *alpha*, *beta and gammaherpesvirinae* [1,3,7–12] as discussed in greater detail below.

Although HHV-6A and HHV6B viral genome integration occurs in several distinct chromosomes, it invariably takes place within the telomeric region of the host chromosomes. The precise mechanism that facilitates integration is still to be defined; however, the presence of TMR at the ends of the viral genomes suggests that these sequences are involved by directing integration into host telomeres. A role for the U94 protein in HHV-6A and HHV-6B integration has been proposed, but remains to be proven experimentally. The relatively wide tropism of HHV-6A and HHV-6B suggest that integration can take place in many different cell types, including gametes. Integration into gametes results in individuals carrying a copy of the HHV-6 genome in every cell of their body. This condition is referred to as inherited chromosomally integrated HHV-6 (iciHHV-6) and is quite common as it is observed in approximately 1% of the human population (50–70 million individuals) worldwide. iciHHV-6 should be distinguished from the commonly used term ciHHV-6 that refers to the presence of integrated HHV-6 genomes, regardless if this is inherited or not. Individuals with iciHHV-6 will transmit the integrated HHV-6 genome according to the Mendelian laws, meaning that 50% of the descendants will inherit iciHHV-6. In this review, the biology of viral integration, the possible medical consequences associated with iciHHV-6 and priority research areas will be discussed.

What is known about HHV-6 latency? Is integration the default mechanism for genome maintenance during latency for HHV-6?

One hallmark of all herpesviruses is that they not only replicate in the infected host but also establish a lifelong persistent infection termed latency. Latency is characterized by the continued presence of the viral genome in infected target cells but the absence of infectious virus production. HHV-6 has been shown to establish a latent infection in various cell types including early bone marrow progenitor cells [13], primary monocytes/macrophages [14], myeloid cell lines [15], an astrocytoma cell line [16] and an oligodendrocyte cell line [17]. In most of these cell types, HHV-6 genes involved in lytic replication are not express and the virus can reactivate, suggesting that it is a quiescent rather than an abortive infection. The target cells differ between HHV-6A and HHV-6B, but more work needs to be done to define the true latency reservoir of both viruses.

Figure 1

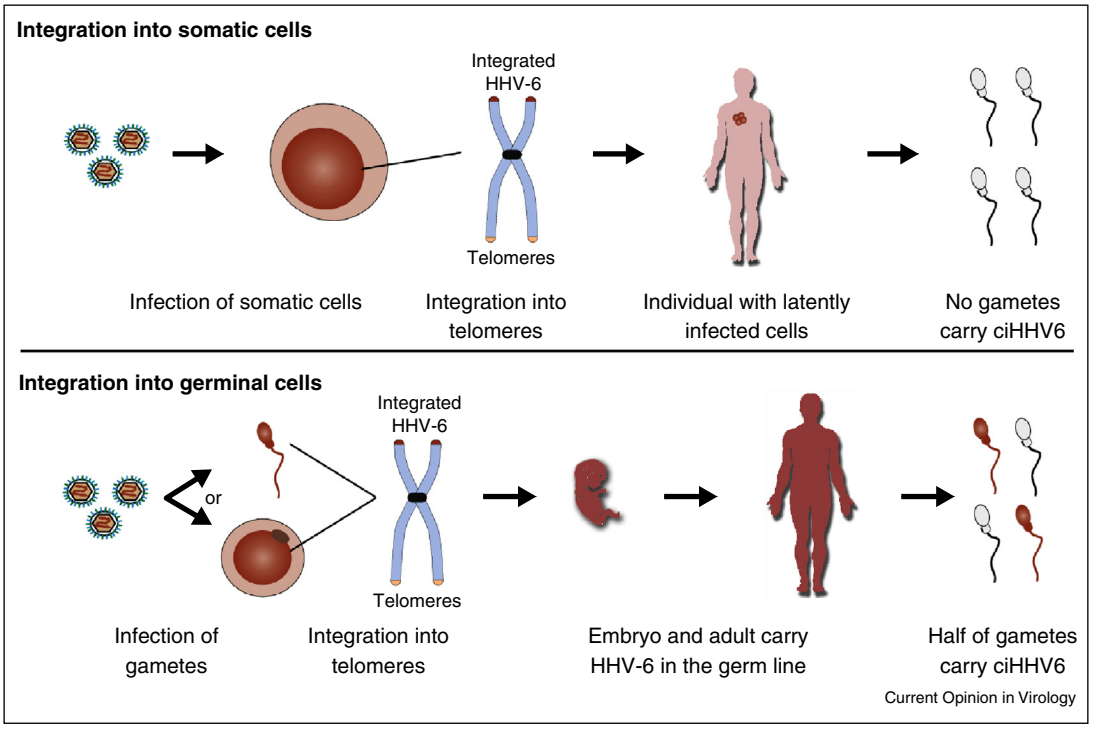


Schematic representation of the HHV-6 genome. The unique region (U) of the HHV-6 genome (140 kbp) is flanked by two identical direct repeat sequences (10–13 kbp) referred to as DR_L and DR_R. The DRs contain pac1 and pac2 sequences, perfect (TMR) and imperfect (impTMR) telomeric sequences (TMR) and several open reading frames (not shown). The genome is not drawn to scale.

During latency, a limited number of transcripts are expressed. Four latency-associated transcripts encoded from the HHV-6 IE1/IE2 locus, are highly spliced and only expressed in latently infected cells *in vitro* and *in vivo* [18]. It has been proposed that these transcripts give rise to three latency-associated proteins termed ORF99, ORF142, and ORF145; however no experimental evidence that confirms their expression is available yet. Furthermore, U94 has been shown to be expressed during latency, even at higher levels compared to lytically infected cells [19]. The U94 protein also blocks viral gene expression in infected lymphocytes in culture [19,20], suggesting that U94 is involved in the establishment and/or maintenance of latency.

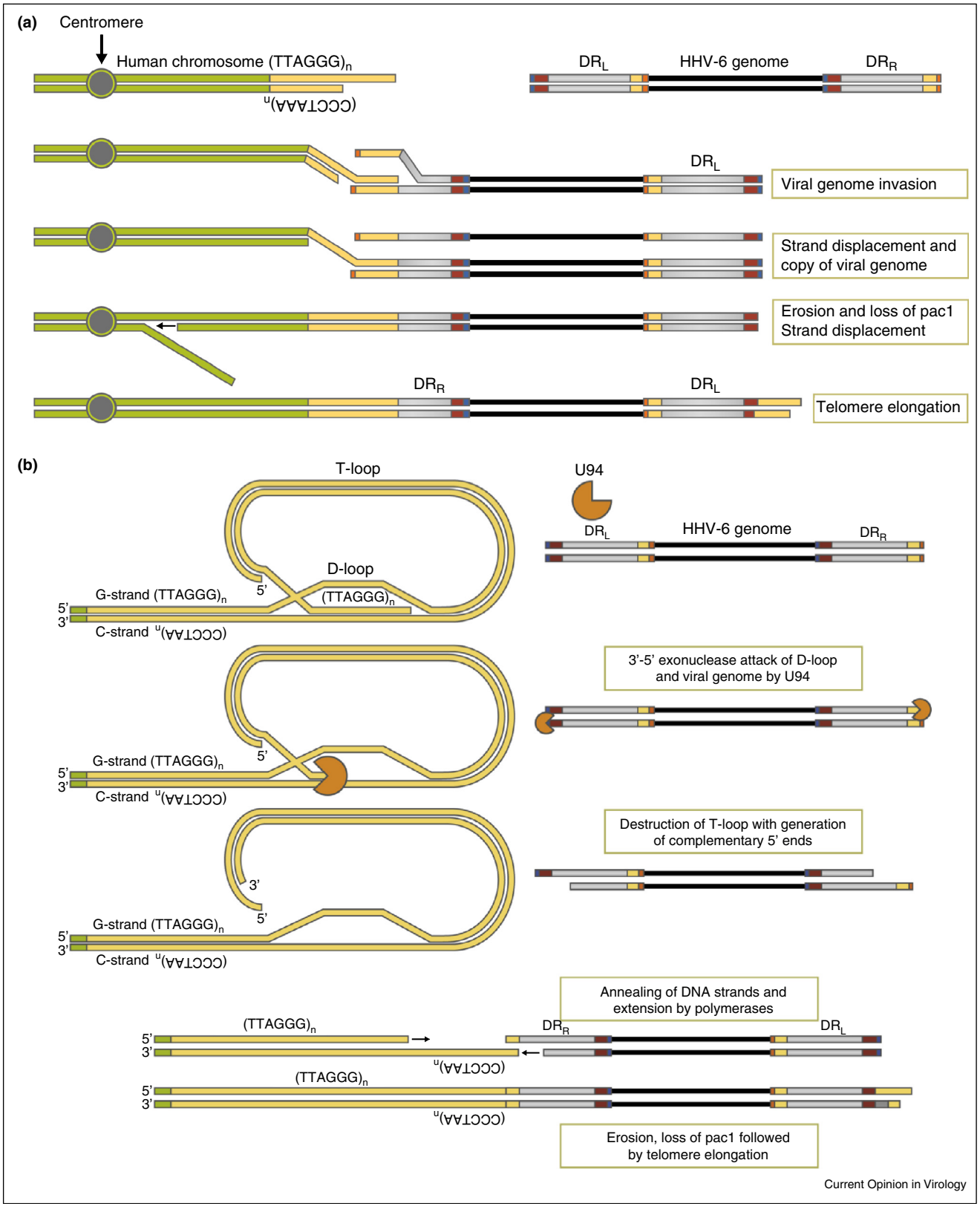
As mentioned above, HHV-6 has been shown to integrate its genome into host telomeres of latently infected cells (Figure 2). Integration of HHV-6 also occurs upon infection of various cell lines including JJhan and Molt-3 T-cells [21••,22], the human embryonic kidney cell line HEK293 [21••,23]. Integration of HHV-6 is not a dead end, as virus reactivation can be induced in cells that harbor the integrated virus genome using the HDAC inhibitor trichostatin A (TSA) or tetradecanoylphorbol-acetate (TPA) [14]. While most herpesviruses maintain their genome as a circular episome in latently infected cells, no episomal copies of the HHV-6 genome were detected [21••,22]. Since only the integrated form of the HHV-6 genome is present during latency, it is likely that integration is the default mechanism for genome maintenance in this phase

Figure 2



Integration of HHV-6 into chromosomes of somatic and germinal cells. During primary HHV-6 infection early in life, the virus infects somatic cells such as monocytes, macrophages and T-cells (upper panel). HHV-6 is able to integrate its genome and establish latency in these cells. Some latently infected cells remain in the host for life, but the virus is not transmitted via the germ line. In addition, HHV-6 is able to infect germinal cells (lower panel). Integration of HHV-6 into sperm cells or oocytes can result in an individual that harbors the virus in the germ line. These individuals pass on iciHHV-6 to 50% of their offspring according to the Mendelian laws of inheritance.

Figure 3



Hypothetical models leading to HHV-6 genome integration into host chromosomes. **(a)** A model based on break-induced homologous recombination (BIR) would allow invasion of 3′ end of the chromosome into the viral genome at the DR_R TMR, followed by stand displacement and copying of the viral

of infection; however, further work needs to be done to determine whether latency can be achieved without integration, i.e. as a viral episome, to decipher the integration mechanism and to understand how the virus genome is mobilized during reactivation.

What is the underlying mechanism of integration? Which cellular and viral factors are potentially involved in the process?

The termini of eukaryotic chromosomes consist of conserved structures termed telomeres that protect the genetic information from terminal deterioration. Vertebrate telomeres consist of 7–10 kb hexameric repeats $(TTAGGG)_n$ that are associated with a number of proteins. During DNA replication, the terminal portion of the telomeres is not completely copied due to the end replication problem. To counteract this shortening, certain cell types express the telomerase complex, which extends the telomeres by the addition TMR sequences to the terminus.

Intriguingly, the HHV-6A and HHV-6B genomes contain TMR at their termini (Figure 1). The fact that HHV-6A and HHV-6B integration invariably occurs in the telomeric region of human chromosomes, suggest that homologous recombination (HR) events between host and viral TMR could facilitate integration.

Chromosome ends have a 3′ single-stranded G-rich (TTAGGG) overhang that is 30–500 nucleotides in length [24–26]. To avoid recognition as double-stranded DNA (dsDNA) break, the 3′ protruding end folds back and invades the duplex telomeric DNA to generate a T-loop structure [27–29]. A total of six proteins referred to as the shelterin complex, bind and assist with T-loop formation, stabilize chromosomal ends and prevent DNA damage responses [30]. Sequencing of the HHV-6 integration sites indicated that the DR_R region of the viral genome is fused to the chromosome [31••]. The pac2 region at this extremity is lost during the integration process. In addition, the pac1 sequences at the other end of the viral genome (DR_L) are also lost and the viral TMR are extended with TTAGGG repeats [31••].

One model for HHV-6 integration compatible with this structure is based on the DNA repair mechanism referred to as break-induced replication (BIR). BIR is a HR pathway that facilitates the repair of DNA breaks that have only one end, contributing to the repair of broken replication forks and allowing telomere lengthening in the absence of telomerase. BIR has been described in various organisms including viruses, bacteria, and eukaryotes (reviewed in [32]). In the context of HHV-6 integration, BIR could be initiated by invasion of the 3′ single strand chromosomal end into the dsDNA linear HHV-6 genome at the TMR sites, followed by DNA synthesis that would continue to the end of the viral genome (Figure 3a). Upon cell replication and division, the 26–28 nucleotide pac1 sequences could erode until the TMR region is encountered. TMR could then serve as template for telomerase, as recently reported [33]. It remains unknown which cellular/viral proteins could participate in this BIR-dependent HHV-6 integration. Considering that BIR is a process that can occur independently of infection, one would argue that viral proteins are dispensable. If HHV-6 integration occurs through BIR, other herpesviruses that possess TMR could use the same mechanism to insert their genome into telomeres of host chromosomes.

A second model for HHV-6 integration is based on the putative integrase HHV-6 U94. U94 encodes a 490 amino acid protein and is unique to HHV-6A and HHV-6B. It has homology (24% identity) to the Adeno-Associated parvovirus (AAV) Rep78/68, a non-structural protein that is essential for AAV integration into chromosomes 19 [34–36]. Besides the similarity, U94 contains the conserved domains of Rep78/68 including the DNA binding and endonuclease domain at the N-terminus as well as the helicase and ATPase domains at the C-terminus [22]. Considering that Rep78/68 is essential for AAV integration and that U94 expression can complement an AAV Rep78/68 deletion mutant [37] suggests that U94 may play a role in HHV-6 integration. U94 possesses single-stranded DNA binding activity [20,38,39] and interacts with the TATA-binding protein [39]. Recent studies indicate that U94 binds telomeric DNA sequences and behaves as a 3′–5′ exonuclease (Trempe and Flamand, unpublished data). A model for U94-dependent integration of HHV-6 into human telomeres is proposed in Figure 3b. Through its DNA-binding and exonuclease activities, U94 would interact with the D-loop structure and remove the protected invading chromosomal end. This would result in the disruption of the T-loop structure and the generation of a 3′ recessed chromosome end. Simultaneously, U94 would attack the HHV-6 genome from its extremities generating a 5′ overhang at the DR_R that is complementary to that of the chromosome. These strands would anneal and polymerases/ligases would fill and close the gaps. As described for the BIR integration, the pac1 at the DR_L end would be lost by erosion followed by telomeric extension using the viral TMR as template.

(Figure 3 Legend Continued) genome. The terminal pac1 sequence would be lost by erosion and the adjacent TMR could be used to restore telomere sequences at the end. This integration process could occur independently of viral proteins. **(b)** U94-dependent integration process. Through its 3′–5′ exonuclease activity, U94 could process the ends of the viral genome and the telomeric D-loop structure, causing the T-loop structure to unfold. This would generate compatible ends that could facilitate annealing of the virus genome and the host chromosome. Upon annealing, the strands would be completed by cellular polymerases/ligases. The terminal pac1 sequence would be lost by erosion and the adjacent TMR could be used to restore telomere sequences at the end.

Do the HHV-6 telomeric repeats facilitate integration into host telomeres? Are other herpesviruses that harbor TMRs able to integrate into the host genome?

As mentioned above, the HHV-6 genome harbors two TMR arrays within the DR regions: the perfect TMR at the right end and the imperfect TMR at the left end of the DR (Figure 1). The number of TMRs varies from 15 to 180 copies in clinical isolates [3,6,9,40]. It has been proposed that the TMR are involved in HHV-6 integration; however, no experimental evidence has been published yet. Deletion of the TMR in the HHV-6 genome resulted in a virus that replicates comparable to parental and revertant viruses (Wallaschek and Kaufer, unpublished data), indicating that the TMR are dispensable for lytic replication. Integration analyses of recombinant viruses that lack the TMRs are currently under investigation.

Besides Marek's disease virus (MDV) and HHV-6, a number of other herpesviruses harbor TMRs [41]. Among them are more than a dozen herpesviruses from the *Herpesviridae* and *Alloherpesviridae* family. These include members of the *alphaherpesvirinae* subfamily such as MDV, herpesvirus of turkeys and duck enteritis virus, the betaherpesviruses HHV-6A, HHV-6B and human herpesvirus 7 (HHV-7) as well as the gammaherpesvirus equine herpesvirus 2. Even the distantly related alloherpesviruses cyprinid herpesvirus 1–3 that infect various fish species including carp, gold fish and koi, harbor TMR at both ends of their genome. The conserved nature of the TMR in various herpesviruses suggests an important function of those repeat sequences. Integration into host telomeres was so far only shown for MDV, HHV-6A and HHV-6B [21••,23,42•], but it is likely that also other herpesvirus containing TMRs in their genome integrate their genetic material. It was recently shown that the viral TMRs can facilitate integration of the virus genome into host telomeres using MDV as a model for herpesvirus integration *in vitro* and *in vivo* [42•]. As HHV-6A and HHV-6B also integrate into telomeres it is very likely that the viral TMRs are also involved in this process as proposed for the two integration models above. The closely related HHV-7 has not been reported to integrate into host chromosomes so far. In contrast to HHV-6, HHV-7 has a very narrow tropism as it infects only CD4 expressing cells [43]. Since only few cells are latently infected with HHV-7, it is very difficult to identify these cells to determine the status of the virus genome within an individual. Intriguingly, HHV-7 does not encode a homologue of U94, suggesting that this protein might be the decisive factor for integration.

Another open question is whether germ line integration also occurs with herpesviruses other than HHV-6A and HHV-6B. In case of HHV-7, the virus likely does not infect gametes as they do not express CD4, providing a possible explanation why germ line integration was not observed for this herpesvirus so far. A recent report demonstrated that tarsier monkeys carry an endogenous herpesvirus closely related to HHV-6 in their genome termed Tarsius syrichta roseolovirus 1 [44]. Unlike HHV-6, the genome of the tarsier monkey virus contains several mutations raising doubts that functional viruses could reactivate from the integrated state. Future studies should address if other herpesviruses that harbor telomeres are also able to integrate into the germ line.

Inherited chromosomally integrated HHV-6: major issues and top research priorities

Undoubtedly, the ultimate question is whether iciHHV-6 represents a risk factor in disease development. It is now well established that the self-renewal potential of cells is directly linked to telomere length and telomerase activity [45,46]. It is also known that the shortest telomere, not average telomere length, is critical for cell viability and chromosome stability [47]. Recent work by Huang et al. indicates that chromosomes carrying integrated HHV-6 often have the shortest telomeres [31••]. Once the number of telomeric repeated sequence (TMR) is reduced to 13, chromosomal instability is observed [48]. Several diseases are linked to telomere dysfunctions and/or telomerase mutations such as hematopoietic dysfunction, pulmonary fibrosis, liver disease, degenerative diseases and cancer [49–59]. Alterations within telomeric regions are therefore a likely cause for cellular dysfunctions linked to diseases. Intriguingly, Pellett et al. reported that iciHHV-6 is 2.3× more frequent ($P < 0.001$) in diseased (various diseases) individuals relative to healthy ones [60]. One potential caveat of this study is that the data was pooled from several small independent studies. The fact that the prevalence of iciHHV6+ individual varies between 0.2% and 2.9% depending on the geographical regions and population sampled (healthy versus diseased) likely affected the outcome of the study. Sampling of a large cohort (n = 50 000 subjects) of individuals aged over 40, when the prevalence of disease is greater, and from a region where the population is relatively homogenous (to minimize confounding factors) would represent an almost ideal way to address the clinical aspects of iciHHV-6. Access to medical records is key for such analyses. By comparing the prevalence of specific diseases in iciHHV-6 versus non-iciHHV-6 individuals, risk factors could be estimated. Once identified, more precise questions pertaining to the mechanisms causing disease could be addressed. In addition, integration of the virus in non-iciHHV-6 patients during latency could also alter cellular function of target cells such as T cells, monocytes, neuroglial cells. This could also influence co-infections with other pathogens or increase the risk of autoimmune disease.

Another area worth investigating is the ability of HHV-6 to infect gametes. To facilitate incorporation into the germ line, HHV-6 must infect gametes and integrate its genome into a host chromosome. This integration most likely occurs in an ovum or sperm progenitor cells, thereby increasing the likelihood transferring the HHV-6 genome into an embryo. Intriguing work by the Hollsberg group indicates that HHV-6 is present in sperm of healthy males and that the virus can bind to sperm cells. This binding mechanism would allow transport of the virus along with the sperm cells to the ovum [61•]. Another possibility is that HHV-6 can reactivate from infected sperm cells and spread to a fertilized egg cell. Whether a haploid chromosome content influences integration remains unknown. Clearly more work on this subject is needed to fully appreciate the initial steps leading to iciHHV-6.

Lastly, the mechanism that allows mobilization of the integrated HHV-6 genome, resulting in reactivation and pathogenesis, remains a fundamental question. Recent studies *in vitro* [21••] and *in vivo* [63•] provided some evidence that integrated HHV-6 can indeed mobilize its genome and reactivate. Two reports suggest that viral excision could occur through the formation of T-circles [31••,62]. These T-circles would arise from recombination events between HHV6 DR regions and result in the generation of a full length circular viral genome containing a single DR. This genome would then serve as template for rolling-circle replication of the virus genome, resulting concatemeric viral DNA. Further convincing evidence was recently provided by Endo et al. that reported pathogenesis from reactivated iciHHV-6A in a Japanese infant with X-SCID [64••]. The profound immunosuppression observed in X-SCID was most likely a key-contributing factor for the observed uncontrolled viral replication. From these observations, two major research priorities emerge. First, in the absence of safe and highly effective anti-HHV-6 drugs, the development of immunotherapeutic approaches to prevent/control HHV-6 reactivation is warranted. HHV-6 specific T cells recognizing peptides derived from the U11, U54 and IE1 proteins have recently been identified [65–68]. Whether these could prevent HHV-6 reactivation and disease should be addressed in a clinical setting. The second research priority relates to the transplantation of organs derived from iciHHV-6+ individuals. Even though almost everyone is already infected with HHV-6, the burden of latently infected cells is likely minimal compared to the number of HHV-6-infected cells introduced upon transfer of organs such as kidneys, livers or even bone marrow cells from an iciHHV-6+ donor. Organs from iciHHV-6+ donors would represent an important reservoir of latently infected cells, possibly reactivating considerable amounts of HHV-6. In addition, cells of iciHHV-6 patients are known to express viral RNAs in the absence of reactivation. Cells expressing HHV-6 proteins would be attacked by the immune system, which could explain, at least in part, idiopathic chronic organ rejection. Realizing that the demand for organ exceeds organ donation, monitoring of iciHHV-6 status of organ donors should be determined prior transplantation to avoid adverse effects and ensure proper diagnosis and treatment by the clinicians.

Conclusion

Initially considered an oddity among virologists, HHV-6 integration is now considered a part of the natural HHV-6 life cycle. Establishment of latency by integration of the virus genome into host chromosomes allows HHV-6 to minimize detection by immune effector cells, ensuring its long-term persistence. It should be pointed out that HHV-6 integration has so far been only observed in iciHHV-6 individuals. The search for cells carrying integrated HHV-6 from a non-iciHHV-6 is still ongoing. Recent advances have started to unravel how these viruses might excise themselves from the integrated state. Although the processes leading to integration remain elusive for the most part, the medical consequences associated with iciHHV-6 are now starting to be recognized as a risk factor for disease development. Large-scale population studies and systemic monitoring of iciHHV-6 status would provide conclusive answers to the biological and medical consequences associated with iciHHV-6.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Martin ME, Thomson BJ, Honess RW, Craxton MA, Gompels UA, Liu MY, Littler E, Arrand JR, Teo I, Jones MD: **The genome of human herpesvirus 6: maps of unit-length and concatemeric genomes for nine restriction endonucleases**. *J Gen Virol* 1991, **72**:157-168.

2. Dominguez G, Dambaugh TR, Stamey FR, Dewhurst S, Inoue N, Pellett PE: **Human herpesvirus 6B genome sequence: coding content and comparison with human herpesvirus 6A**. *J Virol* 1999, **73**:8040-8052.

3. Gompels UA, Macaulay HA: **Characterization of human telomeric repeat sequences from human herpesvirus 6 and relationship to replication**. *J Gen Virol* 1995, **76**:451-458.

4. Isegawa Y, Mukai T, Nakano K, Kagawa M, Chen J, Mori Y, Sunagawa T, Kawanishi K, Sashihara J, Hata A *et al.*: **Comparison of the complete DNA sequences of human herpesvirus 6 variants A and B**. *J Virol* 1999, **73**:8053-8063.

5. Deng H, Dewhurst S: **Functional identification and analysis of cis-acting sequences which mediate genome cleavage and packaging in human herpesvirus 6**. *J Virol* 1998, **72**:320-329.

6. Thomson BJ, Dewhurst S, Gray D: **Structure and heterogeneity of the a sequences of human herpesvirus 6 strain variants U1102 and Z29 and identification of human telomeric repeat sequences at the genomic termini**. *J Virol* 1994, **68**:3007-3014.

7. Buckmaster AE, Scott SD, Sanderson MJ, Boursnell ME, Ross NL, Binns MM: **Gene sequence and mapping data from Marek's disease virus and herpesvirus of turkeys: implications for herpesvirus classification**. *J Gen Virol* 1988, **69**:2033-2042.

8. Kishi M, Bradley G, Jessip J, Tanaka A, Nonoyama M: **Inverted repeat regions of Marek's disease virus DNA possess a structure similar to that of the a sequence of herpes simplex virus DNA and contain host cell telomere sequences**. *J Virol* 1991, **65**:2791-2797.

9. Kishi M, Harada H, Takahashi M, Tanaka A, Hayashi M, Nonoyama M, Josephs SF, Buchbinder A, Schachter F, Ablashi DV *et al.*: **A repeat sequence, GGGTTA, is shared by DNA of human herpesvirus 6 and Marek's disease virus**. *J Virol* 1988, **62**:4824-4827.

10. Secchiero P, Nicholas J, Deng H, Xiaopeng T, van Loon N, Ruvolo VR, Berneman ZN, Reitz MS Jr, Dewhurst S: **Identification of human telomeric repeat motifs at the genome termini of human herpesvirus 7: structural analysis and heterogeneity**. *J Virol* 1995, **69**:8041-8045.

11. Nicholas J: **Determination and analysis of the complete nucleotide sequence of human herpesvirus**. *J Virol* 1996, **70**:5975-5989.

12. Telford EA, Watson MS, Aird HC, Perry J, Davison AJ: **The DNA sequence of equine herpesvirus 2**. *J Mol Biol* 1995, **249**:520-528.

13. Luppi M, Barozzi P, Morris C, Maiorana A, Garber R, Bonacorsi G, Donelli A, Marasca R, Tabilio A, Torelli G: **Human herpesvirus 6 latently infects early bone marrow progenitors in vivo**. *J Virol* 1999, **73**:754-759.

14. Kondo K, Kondo T, Okuno T, Takahashi M, Yamanishi K: **Latent human herpesvirus 6 infection of human monocytes/macrophages**. *J Gen Virol* 1991, **72**:1401-1408.

15. Yasukawa M, Ohminami H, Sada E, Yakushijin Y, Kaneko M, Yanagisawa K, Kohno H, Bando S, Fujita S: **Latent infection and reactivation of human herpesvirus 6 in two novel myeloid cell lines**. *Blood* 1999, **93**:991-999.

16. Yoshikawa T, Asano Y, Akimoto S, Ozaki T, Iwasaki T, Kurata T, Goshima F, Nishiyama Y: **Latent infection of human herpesvirus 6 in astrocytoma cell line and alteration of cytokine synthesis**. *J Med Virol* 2002, **66**:497-505.

17. Ahlqvist J, Fotheringham J, Akhyani N, Yao K, Fogdell-Hahn A, Jacobson S: **Differential tropism of human herpesvirus 6 (HHV-6) variants and induction of latency by HHV-6A in oligodendrocytes**. *J Neurovirol* 2005, **11**:384-394.

18. Kondo K, Shimada K, Sashihara J, Tanaka-Taya K, Yamanishi K: **Identification of human herpesvirus 6 latency-associated transcripts**. *J Virol* 2002, **76**:4145-4151.

19. Rotola A, Ravaioli T, Gonelli A, Dewhurst S, Cassai E, Di Luca D: **U94 of human herpesvirus 6 is expressed in latently infected peripheral blood mononuclear cells and blocks viral gene expression in transformed lymphocytes in culture**. *Proc Natl Acad Sci U S A* 1998, **95**:13911-13916.

20. Caselli E, Bracci A, Galvan M, Boni M, Rotola A, Bergamini C, Cermelli C, Dal Monte P, Gompels UA, Cassai E *et al.*: **Human herpesvirus 6 (HHV-6) U94/REP protein inhibits betaherpesvirus replication**. *Virology* 2006, **346**:402-414.

21. •• Arbuckle JH, Medveczky MM, Luka J, Hadley SH, Luegmayr A, Ablashi D, Lund TC, Tolar J, De Meirleir K, Montoya JG *et al.*: **The latent human herpesvirus-6A genome specifically integrates in telomeres of human chromosomes in vivo and in vitro**. *Proc Natl Acad Sci U S A* 2010, **107**:5563-5568.
First report describing *in vitro* integration and reactivation of HHV-6.

22. Arbuckle JH, Medveczky PG: **The molecular biology of human herpesvirus-6 latency and telomere integration**. *Microbes Infect* 2011, **13**:731-741.

23. Arbuckle JH, Pantry SN, Medveczky MM, Prichett J, Loomis KS, Ablashi D, Medveczky PG: **Mapping the telomere integrated genome of human herpesvirus 6A and 6B**. *Virology* 2013, **442**:3-11.

24. Chai W, Shay JW, Wright WE: **Human telomeres maintain their overhang length at senescence**. *Mol Cell Biol* 2005, **25**:2158-2168.

25. Makarov VL, Hirose Y, Langmore JP: **Long G tails at both ends of human chromosomes suggest a C strand degradation mechanism for telomere shortening**. *Cell* 1997, **88**:657-666.

26. McElligott R, Wellinger RJ: **The terminal DNA structure of mammalian chromosomes**. *EMBO J* 1997, **16**:3705-3714.

27. Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H, de Lange T: **Mammalian telomeres end in a large duplex loop**. *Cell* 1999, **97**:503-514.

28. Nikitina T, Woodcock CL: **Closed chromatin loops at the ends of chromosomes**. *J Cell Biol* 2004, **166**:161-165.

29. Raices M, Verdun RE, Compton SA, Haggblom CI, Griffith JD, Dillin A, Karlseder J: ***C. elegans* telomeres contain G-strand and C-strand overhangs that are bound by distinct proteins**. *Cell* 2008, **132**:745-757.

30. de Lange T: **How telomeres solve the end-protection problem**. *Science* 2009, **326**:948-952.

31. •• Huang Y, Hidalgo-Bravo A, Zhang E, Cotton VE, Mendez-Bermudez A, Wig G, Medina-Calzada Z, Neumann R, Jeffreys AJ, Winney B *et al.*: **Human telomeres that carry an integrated copy of human herpesvirus 6 are often short and unstable, facilitating release of the viral genome from the chromosome**. *Nucleic Acids Res* 2014, **42**:315-327.
Detailed study of the structure of the integrated HHV-6 genome with molecular evidence indicating viral excision from host DNA.

32. Malkova A, Ira G: **Break-induced replication: functions and molecular mechanism**. *Curr Opin Genet Dev* 2013, **23**:271-279.

33. Ohye T, Inagaki H, Ihira M, Higashimoto Y, Kato K, Oikawa J, Yagasaki H, Niizuma T, Takahashi Y, Kojima S *et al.*: **Dual roles for the telomeric repeats in chromosomally integrated human herpesvirus-6**. *Sci Rep* 2014, **4**:4559.

34. Linden RM, Ward P, Giraud C, Winocour E, Berns KI: **Site-specific integration by adeno-associated virus**. *Proc Natl Acad Sci U S A* 1996, **93**:11288-11294.

35. Linden RM, Winocour E, Berns KI: **The recombination signals for adeno-associated virus site-specific integration**. *Proc Natl Acad Sci U S A* 1996, **93**:7966-7972.

36. Thomson BJ, Efstathiou S, Honess RW: **Acquisition of the human adeno-associated virus type-2 rep gene by human herpesvirus type-6**. *Nature* 1991, **351**:78-80.

37. Thomson BJ, Weindler FW, Gray D, Schwaab V, Heilbronn R: **Human herpesvirus 6 (HHV-6) is a helper virus for adeno-associated virus type 2 (AAV-2) and the AAV-2 rep gene homologue in HHV-6 can mediate AAV-2 DNA replication and regulate gene expression**. *Virology* 1994, **204**:304-311.

38. Dhepakson P, Mori Y, Jiang YB, Huang HL, Akkapaiboon P, Okuno T, Yamanishi K: **Human herpesvirus-6 rep/U94 gene product has single-stranded DNA-binding activity**. *J Gen Virol* 2002, **83**:847-854.

39. Mori Y, Dhepakson P, Shimamoto T, Ueda K, Gomi Y, Tani H, Matsuura Y, Yamanishi K: **Expression of human herpesvirus 6B rep within infected cells and binding of its gene product to the TATA-binding protein in vitro and in vivo**. *J Virol* 2000, **74**:6096-6104.

40. Achour A, Malet I, Deback C, Bonnafous P, Boutolleau D, Gautheret-Dejean A, Agut H: **Length variability of telomeric repeat sequences of human herpesvirus 6 DNA**. *J Virol Methods* 2009, **159**:127-130.

41. Osterrieder N, wallascheck N, Kaufer B: **Herpesvirus genome integration into telomeric repeats of host cell chromosomes**. *Annu Rev Virol* 2014, **1** (in press).

42. • Kaufer BB, Jarosinski KW, Osterrieder N: **Herpesvirus telomeric repeats facilitate genomic integration into host telomeres and mobilization of viral DNA during reactivation**. *J Exp Med* 2011, **208**:605-615.

Study demonstrating that herpesvirus TMRs facilitate MDV integration into host telomeres.

43. Lusso P, Secchiero P, Crowley RW, Garzino-Demo A, Berneman ZN, Gallo RC: **CD4 is a critical component of the receptor for human herpesvirus 7: interference with human immunodeficiency virus**. *Proc Natl Acad Sci U S A* 1994, **91**:3872-3876.

44. Aswad A, Katzourakis A: **The first endogenous herpesvirus, identified in the tarsier genome, and novel sequences from primate rhadinoviruses and lymphocryptoviruses**. *PLoS Genet* 2014, **10**:e1004332.

45. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE: **Extension of life-span by introduction of telomerase into normal human cells**. *Science* 1998, **279**:349-352.

46. Harley CB, Futcher AB, Greider CW: **Telomeres shorten during ageing of human fibroblasts**. *Nature* 1990, **345**:458-460.

47. Hemann MT, Strong MA, Hao LY, Greider CW: **The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability**. *Cell* 2001, **107**:67-77.

48. Capper R, Britt-Compton B, Tankimanova M, Rowson J, Letsolo B, Man S, Haughton M, Baird DM: **The nature of telomere fusion and a definition of the critical telomere length in human cells**. *Genes Dev* 2007, **21**:2495-2508.

49. Sahin E, Colla S, Liesa M, Moslehi J, Muller FL, Guo M, Cooper M, Kotton D, Fabian AJ, Walkey C *et al.*: **Telomere dysfunction induces metabolic and mitochondrial compromise**. *Nature* 2011, **470**:359-365.

50. Artandi SE, Chang S, Lee SL, Alson S, Gottlieb GJ, Chin L, DePinho RA: **Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice**. *Nature* 2000, **406**:641-645.

51. Vulliamy T, Marrone A, Dokal I, Mason PJ: **Association between aplastic anaemia and mutations in telomerase RNA**. *Lancet* 2002, **359**:2168-2170.

52. Yamaguchi H, Calado RT, Ly H, Kajigaya S, Baerlocher GM, Chanock SJ, Lansdorp PM, Young NS: **Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia**. *N Engl J Med* 2005, **352**:1413-1424.

53. Wilkie AO, Lamb J, Harris PC, Finney RD, Higgs DR: **A truncated human chromosome 16 associated with alpha thalassaemia is stabilized by addition of telomeric repeat (TTAGGG)$_n$**. *Nature* 1990, **346**:868-871.

54. Mitchell JR, Wood E, Collins K: **A telomerase component is defective in the human disease dyskeratosis congenita**. *Nature* 1999, **402**:551-555.

55. Qazilbash MH, Liu JM, Vlachos A, Fruchtman S, Messner H, Zipursky A, Alter BP, Young NS: **A new syndrome of familial aplastic anemia and chronic liver disease**. *Acta Haematol* 1997, **97**:164-167.

56. Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, Lawson WE, Xie M, Vulto I, Phillips JA 3rd *et al.*: **Telomerase mutations in families with idiopathic pulmonary fibrosis**. *N Engl J Med* 2007, **356**:1317-1326.

57. O'Sullivan JN, Bronner MP, Brentnall TA, Finley JC, Shen WT, Emerson S, Emond MJ, Gollahon KA, Moskovitz AH, Crispin DA *et al.*: **Chromosomal instability in ulcerative colitis is related to telomere shortening**. *Nat Genet* 2002, **32**:280-284.

58. Calado RT, Regal JA, Hills M, Yewdell WT, Dalmazzo LF, Zago MA, Lansdorp PM, Hogge D, Chanock SJ, Estey EH *et al.*: **Constitutional hypomorphic telomerase mutations in patients with acute myeloid leukemia**. *Proc Natl Acad Sci U S A* 2009, **106**:1187-1192.

59. Ohyashiki JH, Ohyashiki K, Fujimura T, Kawakubo K, Shimamoto T, Iwabuchi A, Toyama K: **Telomere shortening associated with disease evolution patterns in myelodysplastic syndromes**. *Cancer Res* 1994, **54**:3557-3560.

60. Pellett PE, Ablashi DV, Ambros PF, Agut H, Caserta MT, Descamps V, Flamand L, Gautheret-Dejean A, Hall CB, Kamble RT *et al.*: **Chromosomally integrated human herpesvirus 6: questions and answers**. *Rev Med Virol* 2012, **22**:144-155.

61. • Kaspersen MD, Larsen PB, Kofod-Olsen E, Fedder J, Bonde J, Hollsberg P: **Human herpesvirus-6A/B binds to spermatozoa acrosome and is the most prevalent herpesvirus in semen from sperm donors**. *PLOS ONE* 2012, **7**:e48810.

First study to document the presence of HHV-6 in sperm fluid of healthy subjects and the ability of HHV-6 to attach to sperm cells.

62. Prusty BK, Krohne G, Rudel T: **Reactivation of chromosomally integrated human herpesvirus-6 by telomeric circle formation**. *PLOS Genet* 2013, **9**:e1004033.

63. • Gravel A, Hall CB, Flamand L: **Sequence analysis of transplacentally acquired human herpesvirus 6 DNA is consistent with transmission of a chromosomally integrated reactivated virus**. *J Infect Dis* 2013, **207**:1585-1589.

Provided *in vivo* evidence of iciHHV-6 reactivation and transplacental transmission.

64. •• Endo A, Watanabe K, Ohye T, Suzuki K, Matsubara T, Shimizu N, Kurahashi H, Yoshikawa T, Katano H, Inoue N *et al.*: **Molecular and virological evidence of viral activation from chromosomally integrated human herpesvirus 6A in a patient with X-linked severe combined immunodeficiency**. *Clin Infect Dis* 2014, **59**:545-548.

Provided the first conclusive evidence of *in vivo* reactivation from integrated HHV-6 with pathogenic outcomes.

65. Gerdemann U, Keukens L, Keirnan JM, Katari UL, Nguyen CT, de Pagter AP, Ramos CA, Kennedy-Nasser A, Gottschalk SM, Heslop HE *et al.*: **Immunotherapeutic strategies to prevent and treat human herpesvirus 6 reactivation after allogeneic stem cell transplantation**. *Blood* 2013, **121**:207-218.

66. Martin LK, Schub A, Dillinger S, Moosmann A: **Specific CD8(+) T cells recognize human herpesvirus 6B**. *Eur J Immunol* 2012, **42**:2901-2912.

67. Nastke MD, Becerra A, Yin L, Dominguez-Amorocho O, Gibson L, Stern LJ, Calvo-Calle JM: **Human CD4+ T cell response to human herpesvirus 6**. *J Virol* 2012, **86**:4776-4792.

68. Iampietro M, Morissette G, Gravel A, Dubuc I, Rousseau M, Hasan AN, O'Reilly RJ, Flamand L: **Human herpesvirus 6B immediate early I protein contains functional HLA-A*02, A*03 and B*07 class I-restricted CD8+ T cell epitopes**. *Eur J Immunol* 2014. PMID: 25243920.

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Current Opinion in Virology

Recent developments in animal models for human herpesvirus 6A and 6B

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Progress in the identification of suitable animal models for human herpesvirus (HHV)-6A and HHV-6B infections has been slow. Recently, new models have been established, mainly for HHV-6A, which reproduce some pathological features seen in humans. Neuroinflammatory signs were observed in infected marmosets and CD46-transgenic mice; although viral replication was not prominent, persistence of viral DNA and specific immunologic responses were detected, suggesting an immune-mediated pathogenic mechanism. Pig-tailed macaques showed robust viral replication concomitant with acute-phase symptoms, and provided a model to study the effects of HHV-6A on AIDS progression. In humanized mice, viral replication was less evident, but infection led to T-cell alterations. Altogether, these recent developments have opened new perspectives for studying the pathogenic role of HHV-6A in humans.

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Introduction

HHV-6A and HHV-6B are members of the betaherpesvirus subfamily, and humans are their only known natural host. HHV-6A and HHV-6B share many properties with other herpesviruses, including the establishment of a persistent latent infection characterized by highly restricted viral gene expression, and the ability to reactivate from latency to produce infectious virus. Although originally categorized as two variants, HHV-6A and HHV-6B were recently re-classified as independent viruses based upon differences in epidemiology, tropism, and disease associations [1]. CD46 serves as the main cellular receptor for HHV-6A [2], while CD134 was recently identified as a novel receptor for HHV-6B [3]. *In vivo* tropism of these viruses includes $CD4^+$ T cells, epithelial cells in salivary glands and liver, endothelial cells, and cells of the central nervous system (CNS) [4]. HHV-6A replicates in neural cells in culture more efficiently than HHV-6B and is thought to be overall more neurotropic [5].

HHV-6B infection is very common in the human population worldwide, with a very high seroprevalence (>90%) by age two [6]. Acute primary HHV-6B infection can result in exanthem subitum [7], a childhood febrile disease accompanied by a rash and, in rare cases, by febrile convulsions. No disease association has been firmly established for HHV-6A, although evidence suggests a role in hematopoietic stem cell and solid organ transplant complications [8], graft-versus-host disease [9], and multiple sclerosis [10,11]. Disease manifestations by both HHV-6A and HHV-6B are often correlated with host immunosuppression, which may promote viral reactivation from latency. The prevalence of HHV-6A infection is still largely undefined due to a lack of serological assays that can clearly distinguish between HHV-6A and HHV-6B infections.

The lack of animal models that efficiently support HHV-6A or HHV-6B replication has long hindered studies of viral pathogenesis. The focus of this review is on recent work aimed at developing new animal models that sustain HHV-6A and/or HHV-6B replication, which may help to better understand the pathogenic mechanisms of these viruses in humans.

New animal models of neuropathology

Marmoset model

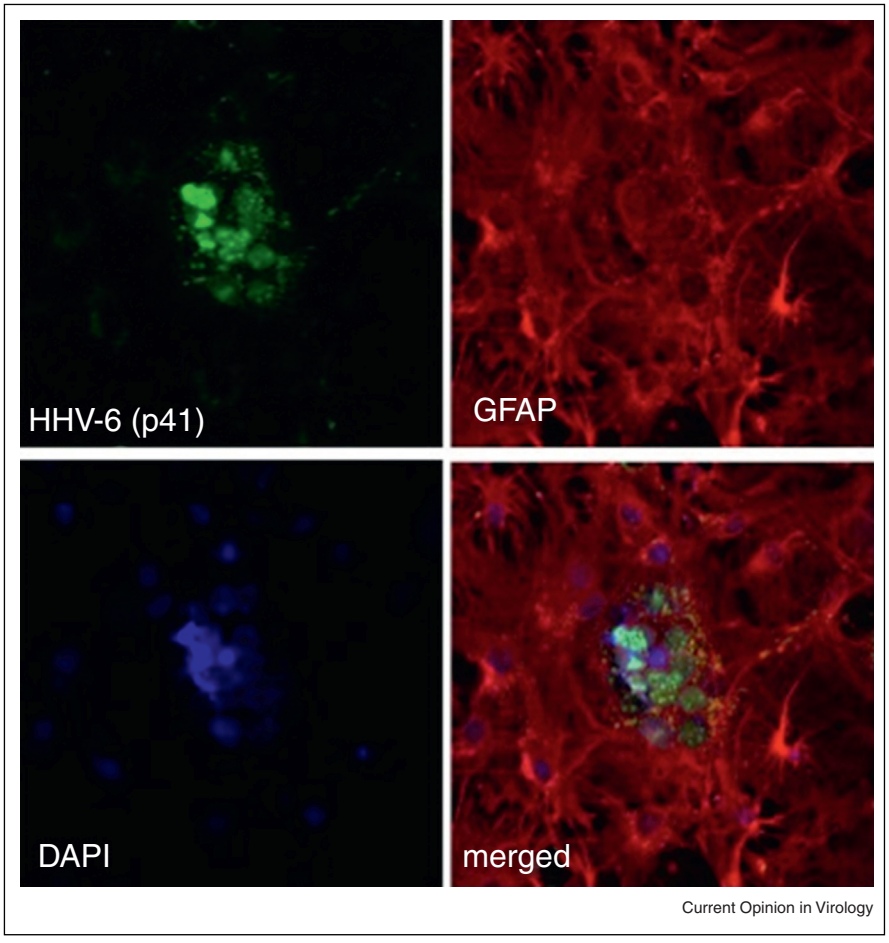
Recently, a marmoset (*Callithrix jacchus*) model was developed to study HHV-6A and HHV-6B infections [12••]. Marmosets that received multiple intravenous injections of HHV-6A developed neurological symptoms, including motor weakness and sensory abnormalities, associated with the development of virus-specific antibody responses and with the presence of histopathological lesions in the CNS, primarily microgliosis. Viral DNA was detected in the brain of HHV-6A-infected and HHV-6B-infected animals, confirming the neurotropism of both viruses. However,

while HHV-6A infection led to evident neurological symptoms, infection with HHV-6B remained asymptomatic. Surprisingly, HHV-6A infection through the intranasal route also remained completely asymptomatic and elicited limited, if any, antibody responses despite detectable levels of plasma viremia. The correlation between the development of neurological signs and the elicitation of virus-specific humoral immune responses in this model suggests a possible immune-mediated pathogenic mechanism rather than a direct neuropathic effect of HHV-6A infection. This study provided the first conclusive *in vivo* evidence that HHV-6A infection is able to trigger neurological disease.

CD46 transgenic mouse model

Since monkey experiments are often limited by ethical constraints and elevated costs, efforts were made in last few years to develop mouse models of HHV-6-associated neuropathology. The human transmembrane complement regulatory protein CD46 was identified as the receptor for HHV-6A entry into host cells [2], opening novel possibilities to develop humanized murine models of HHV-6A infection. Recently, it has been demonstrated that intracranial and intraperitoneal infection of CD46 transgenic mice with HHV-6A results in long-term persistence of viral DNA in the brains of infected animals, followed by lymphocyte infiltration and upregulation of the chemokine CCL5/RANTES, in the absence of clinically apparent signs of disease [13••]. In the presence of HHV-6A-infected human lymphocytes, transgenic murine primary brain cultures were shown to produce viral proteins and develop syncytia (Figure 1); however, viral RNA and proteins have not been detected *in vivo* in mice. Infection with HHV-6B did not yield any signs of viral replication in transgenic murine CD46 transgenic cells either *in vitro* or *in vivo*, probably due to the main utilization of another

Figure 1



HHV-6A infection of primary murine glial brain culture from CD46 trangenic mice. Primary murine brain glial cells generated from CD46-transgenic mice were co-cultured with HHV-6A-infected HSB2 cells as described [13••]. Seven days after the establishment of the co-culture, supernatants and non-adherent cells were removed, and adherent cells were fixed and analyzed for the presence of viral antigens by confocal microscopy. Cells were stained with antibodies against HHV-6 proteins (green) p41 (A-C) or gp116 (D), and glial fibrillary acidic protein (GFAP) antibody (red) and cell nuclei were stained with DAPI (blue).

recently identified entry receptor, CD134 [3]. The secretion of a panel of chemokines was increased after HHV-6A infection of transgenic primary murine brain glial cultures and the induced chemokine expression was inhibited when TLR9 signaling was blocked. These results described the first murine model for HHV-6A-induced brain infection and highlighted the potential importance of the TLR9 pathway in HHV-6A-initiated neuroinflammation, opening novel perspectives for the study of virus-associated neuropathology.

New animal models of immunomodulation and immunodeficiency

Pig-tailed macaque model

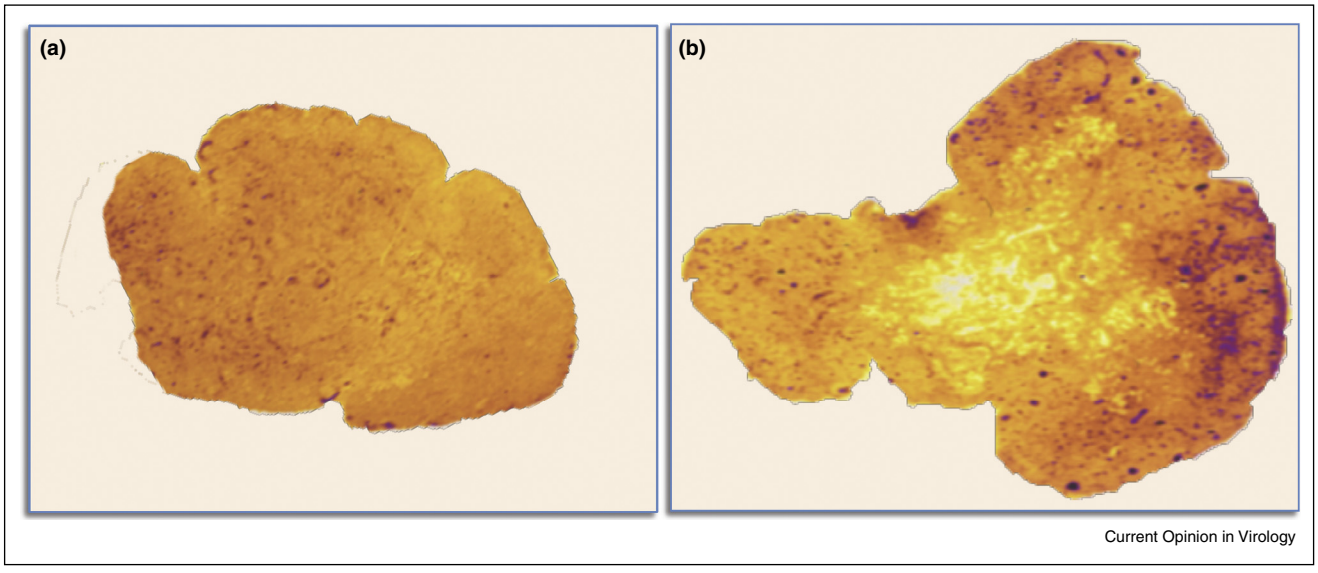
Various non-human primate species have been studied in the past for their susceptibility to HHV-6A, HHV-6B and HHV-7 infections with limited success [14] and (Lusso *et al.*, unpublished), reflecting the inefficient *in vitro* replication of these viruses in primary lymphocytes from the same animals [15]. However, the pig-tailed macaque (*Macaca nemestrina*) was singled out for its ability to sustain HHV-6A replication with human-like efficiency both *in vitro* [16] and, more recently, *in vivo* [17••]. Intravenous inoculation of HHV-6A into naïve pig-tailed macaques resulted in a rapid appearance of plasma viremia and viral RNA transcription in lymph nodes, associated with transient clinical manifestations such as fever, lymphadenopathy and, in one animal, cutaneous rash; IgG antibody seroconversion ensued after approximately 3 weeks of inoculation [17••]. After the acute phase, HHV-6A infection entered a clinically latent state, analogous to observations in healthy children, and no long-term clinical or immunological alterations were detected, except for the occasional finding of transient, low-level plasma viremia. These results reproduced the main virological, immunological, and clinical features of acute HHV-6B infection in humans (to date, no definitive data are available for acute HHV-6A infection), suggesting that pig-tailed macaques may represent a reliable experimental model for these viruses.

The pig-tailed macaque model was also instrumental for investigating the *in vivo* interactions between HHV-6A and simian immunodeficiency virus (SIV), the monkey homologue of human immunodeficiency virus (HIV). Animals co-infected with HHV-6A and SIV showed a dramatic acceleration of SIV-disease progression toward full-blown AIDS, associated with early depletion of both CD4+ and CD8+ T cells and increased SIV expression in lymph nodes (Figure 2); interestingly, as seen in immunodeficient humans, frequent HHV-6A plasma viremia was observed in co-infected animals, concomitant with a progressive deterioration of the host immunologic defenses [17••]. These results provided the first *in vivo* evidence for an accelerating effect of HHV-6A on AIDS progression.

RAG-hu mouse model

Humanized mice are an attractive model for the study of human viral pathogens because they produce human

Figure 2



Enhanced replication of SIV in lymph nodes from HHV-6A-co-infected pig-tailed macaques. *In situ* hybridization in lymph node tissues from macaques singly infected with SIVsmE660 **(A)** or dually infected with SIV and HHV-6A **(B)**. In tissue from the animal singly infected with SIV, the overall architecture is conserved and low levels of SIV RNA (purple signal) are visible throughout the parenchyma, with little, if any, specific signal within reactive germinal centers. In tissue from the dually infected animal, a florid follicular hyperplasia is visible with an intense SIV RNA signal. Co-infection with HHV-6A induced a dramatic acceleration of disease progression toward full-blown AIDS.

target cells and can generate human anti-viral immune responses. In the humanized Rag2$^{-/-}\gamma c^{-/-}$ mouse (RAG-hu) model, human CD34^{+} hematopoietic stem cells are extracted from cord blood or fetal liver and injected into neonatal immunodeficient mice. Engrafted animals produce a variety of human lymphoid and myeloid cells, including CD4^{+} T cells, which are major target cells for HHV-6A.

Recent data show that RAG-hu mice can be infected with HHV-6A following intraperitoneal injection of either cell-free or cell-associated virus, with persistence of viral DNA in blood and lymphoid organs [18••]. Viral DNA was detected only sporadically in plasma and blood cells, possibly due to inefficient replication and establishment of latent infection. The bone marrow was positive for viral DNA in all animals tested at 1 week post-infection. Brain infection has not yet been examined, although human immune cells have been detected in the brain of humanized mice, accompanied by HIV-1 penetration, after peripheral HIV-1 inoculation [19]. Human thymocyte populations were modified after peritoneal inoculation of HHV-6A, indicating cytopathic effects in that organ. The CD3^{+}CD4^{-} and CD3^{-}CD4^{+}CD8^{-} populations were depleted in infected animals (Figure 3). Interestingly, depletion of the CD3^{-}CD4^{+}CD8^{-} thymocyte population had previously been observed in a SCID-hu thy/liv humanized mouse model where HHV-6A or HHV-6B was injected directly into the thymic organoid [20]. A possible contributing mechanism is CD3 downregulation, which has previously been reported in peripheral blood T cells [21] and in cells extracted from lymphoid tissues [22] and is likely mediated by the viral U24 protein [23]. An unusual finding in infected RAG-hu mice was an elevated proportion of CD3^{+}CD4^{+}CD8^{+} T cells in blood, as compared to mock-infected animals. While the origin of these cells is still unclear, HHV-6A infection can promote expression of CD4 on cells that do not normally express it [24]. Thus, it is possible that these cells were either infected with HHV-6A, or triggered to exit the thymus prematurely (most CD4^{+}CD8^{+} cells reside in thymus).

Taken together, the findings in infected RAG-hu mice suggested that HHV-6A has a natural tropism for the human thymus and bone marrow, and that infection leads to alteration of T lymphocyte subpopulations. Depletion and/or alteration of specific thymocyte subsets may play an important role in HHV-6A-induced immunomodulation and the ability of this virus to persist in the host.

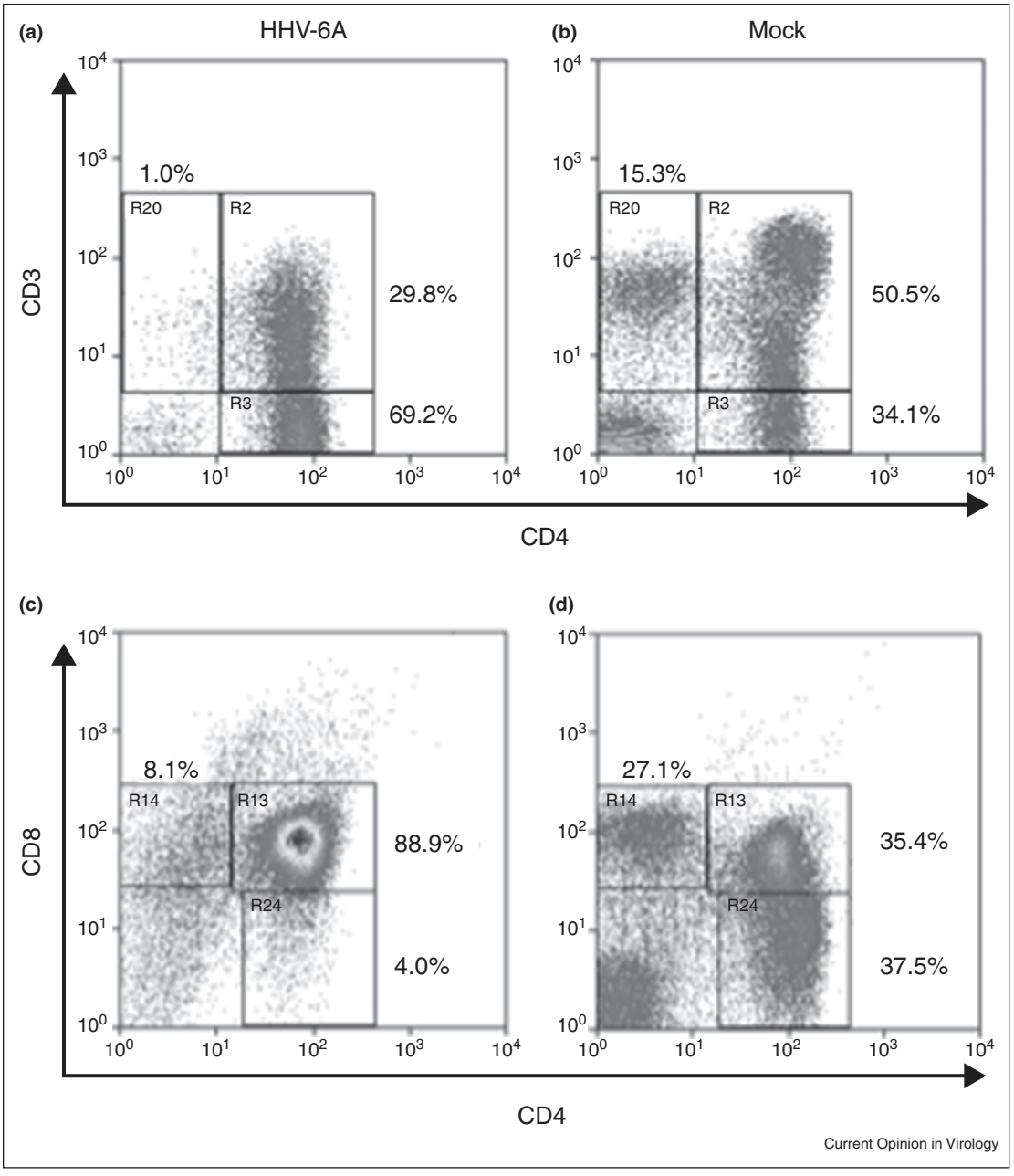
Conclusions and future perspectives

The predominantly latent nature of human roseolovirus infections and our ignorance of the mechanisms that control such viruses *in vivo* have made studies in animal models particularly challenging. Nevertheless, new animal models to study pathology induced by HHV-6A and HHV-6B have been developed during the last decade (Table 1). Although they do not mimic entirely all aspects of the human infections, these models have provided some important insights into the neurological and immunological disorders associated with these viruses. Work in the marmoset model showed neurological symptoms in association with HHV-6A infection. A correlation between HHV-6A infection and multiple sclerosis (MS) has been noted for some time, specifically with increased detection of viral nucleic acids and anti-viral antibody responses in MS patients [25,26]. Further work in the marmoset model may yield additional insights into the role of HHV-6A in this disease whose etiology remains poorly understood. The CD46 transgenic mouse model has further illustrated the potential for neurologic disease associated with HHV-6A in humans by demonstrating that various proinflammatory chemokines are upregulated after infection both *in vivo* and *in vitro* and that immune cells respond to HHV-6A infection in the brain. The TLR9 pathway was identified as a pathway responsible for chemokine upregulation and has been implicated in a different animal model of MS [27], thus providing additional evidence that HHV-6A may be linked to the development of MS.

The recent findings in RAG-hu mice have provided *in vivo* evidence to support a role for HHV-6A in immunosuppression associated with alterations of thymocyte populations. Since the thymus is responsible for T cell development, this may represent a novel mechanism for viral persistence by manipulating T cells before they become functional. The ramifications of thymocyte depletion are currently unclear, but could promote generalized immunosuppression. In addition, macaque studies have provided *in vivo* evidence to support the hypothesis that HHV-6A co-infection leads to more rapid AIDS progression in HIV-infected individuals. Further studies are required to firmly establish a role for HHV-6A in human immunosuppression *in vivo*; however, if a role for this virus in AIDS progression is confirmed, HHV-6A may represent an important new drug target for AIDS treatment.

More animal models have been described for HHV-6A than for HHV-6B infection, possibly reflecting the conservation and ubiquitous distribution of the main HHV-6A receptor, CD46. The recent identification of the immunoregulatory molecule CD134 (OX40), which is expressed predominantly on activated human T cells, as a novel receptor for HHV-6B [3] will certainly lead to the development of additional models for this virus, including transgenic mice. The absence of CD134 expression on CNS cells may explain the apparently lower neurotropism of HHV-6B, compared to HHV-6A; whether and to what

Figure 3



Alteration of thymocyte populations in humanized mice after HHV-6A infection. RAG-hu mice were infected with HHV-6A (or mock infected) and thymocytes were collected at 7.5 wpi and analyzed by flow cytometry. The CD3+CD4− population is depleted in HHV-6A infected animals **(A)** but not in mock infected animals **(B)**. Gating in panels A/B was on lymphocytes, and data were normalized to the sum of gates R2/R3/R20. The CD4+CD8− population is depleted in HHV-6A infected animals (C) but not in mock infected animals (D). Data in panels C/D were not gated and were normalized to the sum of gates R13/R14/R24. Although changes in the CD4−CD8+ and CD4−CD8− populations can be seen in this representative case, these findings were not statistically significant for the entire group of animals.

extent at least some strains of HHV-6B can also utilize CD46 as a receptor, as previously reported [2], remains uncertain. The availability of suitable animal models, especially murine models for which a wide array of experimental tools are available, should facilitate further studies of virus–host interactions and pathogenesis and open novel perspectives for devising effective therapeutic and preventive approaches for HHV-6A and HHV-6B.

Table 1

New animal models recently established to study HHV-6A and HHV-6B pathogenesis

Model [reference]	Virus(es) studied	Route of infection	Major pathologic findings	Disadvantages
Pig-tailed macaques [17••]	HHV-6A	Intravenous	Acute-phase symptoms, robust viral replication, antibody responses, accelerated AIDS progression	Costly, ethical constraints
Marmosets [12••]	HHV-6A and HHV-6B	Intravenous	CNS pathology (HHV-6A only), antibody responses	Low viral replication, costly, ethical constraints
Humanized $Rag2^{-/-}\gamma c^{-/-}$ mice [18••]	HHV-6A	Intraperitoneal	Viral DNA persistence in blood, antibody responses, alteration of human thymocyte and T cell populations	Low viral replication
huCD46-transgenic mice [13••]	HHV-6A	Intracranial + intraperitoneal	Long-term viral DNA persistence in CNS, antibody responses, CNS production of pro-inflammatory cytokines	Low viral replication

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. ICTV: *ICTV Master Species List 2011*. International Committee on Taxonomy of Viruses; 2011:. Available from: http://talk.ictvonline.org/files/ictv_documents/m/msl/4090.aspx [accessed 17.07.14].
2. Santoro F *et al.*: **CD46 is a cellular receptor for human herpesvirus 6**. *Cell* 1999, **99**:817-827.
3. Tang H *et al.*: **CD134 is a cellular receptor specific for human herpesvirus-6B entry**. *Proc Natl Acad Sci U S A* 2013, **110**:9096-9099.
4. Campadelli-Fiume G, Mirandola P, Menotti L: **Human herpesvirus 6: an emerging pathogen**. *Emerg Infect Dis* 1999, **5**:353-366.
5. De Bolle L *et al.*: **Quantitative analysis of human herpesvirus 6 cell tropism**. *J Med Virol* 2005, **75**:76-85.
6. Okuno T *et al.*: **Seroepidemiology of human herpesvirus 6 infection in normal children and adults**. *J Clin Microbiol* 1989, **27**:651-653.
7. Yamanishi K *et al.*: **Identification of human herpesvirus-6 as a causal agent for exanthem subitum**. *Lancet* 1988, **1**:1065-1067.
8. Le J, Gantt S, A.I.D.C.o. Practice: **Human herpesvirus 6, 7 and 8 in solid organ transplantation**. *Am J Transplant* 2013, **13**:128-137.
9. Agut H: **Deciphering the clinical impact of acute human herpesvirus 6 (HHV-6) infections**. *J Clin Virol* 2011, **52**:164-171.
10. Behzad-Behbahani A *et al.*: **Human herpesvirus-6 viral load and antibody titer in serum samples of patients with multiple sclerosis**. *J Microbiol Immunol Infect* 2011, **44**:247-251.
11. Virtanen JO *et al.*: **Evidence for human herpesvirus 6 variant A antibodies in multiple sclerosis: diagnostic and therapeutic implications**. *J Neurovirol* 2007, **13**:347-352.
12. •• Leibovitch E *et al.*: **Novel marmoset (*Callithrix jacchus*) model of human herpesvirus 6A and 6B infections: immunologic, virologic and radiologic characterization**. *PLoS Pathog* 2013, **9**:e1003138.
This paper described the new marmoset model of HHV-6A-induced neuropathology
13. •• Reynaud JM *et al.*: **Human herpesvirus 6A infection in CD46 transgenic mice: viral persistence in the brain and increased production of proinflammatory chemokines via toll-like receptor 9**. *J Virol* 2014, **88**:5421-5436.
This paper described the new transgenic mouse model of HHV-6A-induced neuropathology
14. Yalcin S *et al.*: **Experimental infection of cynomolgus and African green monkeys with human herpesvirus 6**. *J Gen Virol* 1992, **73**:1673-1677.
15. Lusso P *et al.*: **In vitro susceptibility of T lymphocytes from chimpanzees (Pan troglodytes) to human herpesvirus 6 (HHV-6): a potential animal model to study the interaction between HHV-6 and human immunodeficiency virus type 1 in vivo**. *J Virol* 1990, **64**:2751-2758.
16. Lusso P, Secchiero P, Crowley RW: **In vitro susceptibility of Macaca nemestrina to human herpesvirus 6: a potential animal model of coinfection with primate immunodeficiency viruses**. *AIDS Res Hum Retroviruses* 1994, **10**:181-187.
17. •• Lusso P *et al.*: **Human herpesvirus 6A accelerates AIDS progression in macaques**. *Proc Natl Acad Sci U S A* 2007, **104**:5067-5072.
This paper described the new macaque model of HHV-6A acute infection and acceleration of SIV immunodeficiency
18. •• Tanner A *et al.*: **Human herpesvirus 6A infection and immunopathogenesis in humanized $Rag2^{-/-\gamma c-/-}$ mice**. *J Virol* 2013, **87**:12020-12028.
This paper described the new mouse model of HHV-6A-induced immune modulation/deficiency
19. Berges BK, Rowan MR: **The utility of the new generation of humanized mice to study HIV-1 infection: transmission, prevention, pathogenesis, and treatment**. *Retrovirology* 2011, **8**:65.
20. Gobbi A *et al.*: **Human herpesvirus 6 (HHV-6) causes severe thymocyte depletion in SCID-hu Thy/Liv mice**. *J Exp Med* 1999, **189**:1953-1960.
21. Lusso P *et al.*: **Productive infection of CD4+ and CD8+ mature human T cell populations and clones by human herpesvirus 6. Transcriptional down-regulation of CD3**. *J Immunol* 1991, **147**:685-691.
22. Grivel JC *et al.*: **Pathogenic effects of human herpesvirus 6 in human lymphoid tissue ex vivo**. *J Virol* 2003, **77**:8280-8289.

23. Sullivan BM, Coscoy L: **Downregulation of the T-cell receptor complex and impairment of T-cell activation by human herpesvirus 6 u24 protein**. *J Virol* 2008, **82**:602-608.

24. Lusso P *et al.*: **Induction of CD4 and susceptibility to HIV-1 infection in human CD8+ T lymphocytes by human herpesvirus 6**. *Nature* 1991, **349**:533-535.

25. Goodman AD *et al.*: **Human herpesvirus 6 genome and antigen in acute multiple sclerosis lesions**. *J Infect Dis* 2003, **187**:1365-1376.

26. Soldan SS *et al.*: **Association of human herpes virus 6 (HHV-6) with multiple sclerosis: increased IgM response to HHV-6 early antigen and detection of serum HHV-6 DNA**. *Nat Med* 1997, **3**:1394-1397.

27. Prinz M *et al.*: **Innate immunity mediated by TLR9 modulates pathogenicity in an animal model of multiple sclerosis**. *J Clin Invest* 2006, **116**:456-464.

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Current Opinion in Virology

Clinical impact of primary infection with roseoloviruses

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The roseoloviruses, human herpesvirus-6A -6B and -7 (HHV-6A, HHV-6B and HHV-7) cause acute infection, establish latency, and in the case of HHV-6A and HHV-6B, whole virus can integrate into the host chromosome. Primary infection with HHV-6B occurs in nearly all children and was first linked to the clinical syndrome roseola infantum. However, roseolovirus infection results in a spectrum of clinical disease, ranging from asymptomatic infection to acute febrile illnesses with severe neurologic complications and accounts for a significant portion of healthcare utilization by young children. Recent advances have underscored the association of HHV-6B and HHV-7 primary infection with febrile status epilepticus as well as the role of reactivation of latent infection in encephalitis following cord blood stem cell transplantation.

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Introduction

Roseoloviruses include human herpesvirus-6A, -6B and -7 (HHV-6A, HHV-6B and HHV-7), which constitute the *Betaherpesviridae* subfamily of human herpesviruses along with human cytomegalovirus (HCMV). HHV-6 was first isolated from immunocompromised adults in 1986 by Salahuddin and colleagues [1]. Initially two distinct variants of HHV-6 were identified, HHV-6A and B with HHV-6B causing disease in developed countries. The two variants were officially classified as separate viruses in 2012 [2].

As with all human herpesviruses, following primary infection HHV-6 and -7 establish latent or persistent infection in different cell types, have the ability to reactivate, and may be intermittently shed in bodily fluids [3]. Unlike other human herpesviruses, HHV-6A and HHV-6B are also found integrated into the host genome (ciHHV-6). Integration has been documented in 0.2–1% of the general population and along with latency has confounded the ability to correlate the presence of viral nucleic acid with active disease [4].

The syndrome known as roseola infantum was reported as early as 1809 by Robert Willan in his textbook 'On cutaneous diseases' [5]. This clinical entity is also commonly referred to as exanthem subitum and early published descriptions of the disease still hold true. It is an illness that affects children by the age of three and is marked by the abrupt development of high fever lasting three to five days. The hallmark maculopapular rash appears as the fever subsides, and there may be few, if any, associated symptoms. Despite knowledge of this common disease of infancy, the etiologic agent was not identified until 1988 by Yaminishi and colleagues [6]. They demonstrated both the presence of circulating virus in peripheral blood mononuclear cells (PBMCs) during acute roseola and subsequent seroconversion during convalescence in four infants in Japan. It was nearly a decade later before our understanding of the full clinical spectrum of HHV-6 primary infection was expanded past roseola.

Recognition of primary infection with HHV-6 is important because the high prevalence of infection and its association with fever leads to substantial healthcare utilization. Primary infection in childhood is also strongly associated with neurologic complications, and reactivation of the latent virus under immunosuppressive conditions has been associated with significant morbidity. This review discusses the spectrum of clinical disease associated with roseolovirus primary infection, highlighting recent advances.

Epidemiology

The ubiquitous nature of infection with HHV-6 is evidenced by the fact that all newborns have passive maternal antibody to HHV-6 which typically wanes by four to six months of age, with primary infection occurring fairly soon thereafter [7–9]. The young age of primary HHV-6 infection was demonstrated in a prospective study by Hall and colleagues of children with fever seen in the emergency department (ED) in Rochester, NY [7]. Utilizing viral isolation and seroconversion, HHV-6B was identified as the causative agent of illness in 159 of 1553 children less than 24 months of age, while

only one child out of 100 at 25–36 months of age had fever due to primary HHV-6B infection. The peak age of infection was six to nine months [7]. Zerr and colleagues conducted a population-based prospective cohort study of HHV-6 primary infection in children from birth through two years of age in Seattle, WA. On the basis of persistent shedding of HHV-6B DNA in saliva, they noted a peak incidence of primary infection from nine to 21 months of age among children in the community, which is slightly older than the ED-based study. This shift in age of acquisition is also reflected in a 40% cumulative incidence of infection by 12 months of age, but the vast majority of children (77%) still acquired the virus by 24 months of age [10].

While HHV-6A DNA has been identified in umbilical cord blood mononuclear cells and in approximately one third of individuals with ciHHV-6, its role in subsequent active disease has not yet been established [11]. Clinical disease in North America, Europe and Asia has almost exclusively been linked to HHV-6B infection [2]. This contrasts with one region of sub-Saharan Africa, where HHV-6A DNA was detected in a majority of infants in an HIV-1 endemic region [12].

Transmission

The exact modes of transmission of HHV-6 have yet to be definitely determined. It is presumed that HHV-6 can be transmitted from the saliva of asymptomatic adults and older children because of the rapid and reliable transmission of virus to susceptible infants and the lack of recognized outbreaks [3]. It does seem clear that close contact is required for transmission, supported by the observations that having older siblings and parents who share saliva are associated with virus acquisition, but attending daycare is not [10,13]. Recently, transmission of HHV-6 via respiratory droplets has been suggested by the identification of viral DNA in nasal mucosa and olfactory bulb specimens. Olfactory-ensheathing cells, specialized glial cells present in the nasal cavity, are also capable of being infected *in vitro* with HHV-6A suggesting that the olfactory pathway may be a route of entry of HHV-6 into the CNS [14].

Congenital infection with HHV-6 also occurs in approximately 1% of newborns [11]. While this rate is similar to congenital transmission of CMV, 86% of congenital infections are transmitted via chromosomally-integrated virus (ciHHV-6) while a minority (14%) is transmitted through presumed transplacental infection [15]. Chromosomal integration with germline transmission is a mechanism unique to HHV-6 and has not been demonstrated for HHV-7 or any other human herpesvirus. Infants with ciHHV-6 have measurable HHV-6-specific antibody, but it is unknown whether this is protective, whether the virus is actively replicating and the long term effects of congenitally-acquired HHV-6 [4,15,16•].

Clinical presentation

Symptoms

The most common finding in children with HHV-6 primary infection is fever (Table 1). Compared to other febrile illnesses in children under two years of age evaluated in an ED setting, HHV-6 infection has been shown to cause a significantly higher mean temperature (39.6 °C compared to 38.9 °C), with the great majority of children exhibiting temperatures greater than 39 °C. In the study in Rochester NY, fevers remained high for the first three days with 15% of children remaining febrile for six or more days. Children with primary HHV-6 infection also presented earlier into the illness for medical care than children with other febrile illnesses (2.1 versus 2.9 days) [7].

While studies from Japan have strongly linked HHV-6 to the clinical syndrome of roseola, this may be a reflection of study design and subject inclusion criteria [6,17]. Prospective studies in the US have revealed that the classic syndrome of roseola accompanies only a minority of primary HHV-6B infections. The hallmark rash of roseola was observed in only 6% of the children at initial presentation when febrile and in another 17% at the time of defervescence in the study by Hall and colleagues [7]. Similarly, rash was only present in approximately 20% of children during primary HHV-6 infection in the community based study in Seattle, WA [10]. This highlights that roseola infantum is identified in less than a quarter of children with primary HHV-6 infection in the United States.

Fever, fussiness and rhinorrhea are present in over half of children with primary HHV-6B infection while diarrhea, rash and roseola are all significantly more common during primary HHV-6B infection than other periods of illness [10]. Additionally, febrile children with HHV-6B infection are less likely to present with cough or other symptoms of lower respiratory tract infection [7].

Healthcare utilization

HHV-6B primary infection is a common cause of acute medical care visits accounting for 10% of physician office visits and 10–17% of acute febrile ED visits in children up to 36 months of age [7,10,18,19••]. Remarkably, primary infection has been identified in 24% of children from six to nine months of age presenting to the ED with an acute febrile illnesses (Figure 1) [7]. Additionally, children with primary HHV-6B infection are more likely to present with signs of serious systemic illness, irritability, and inflamed tympanic membranes and are commonly diagnosed with a presumed serious bacterial infection or otitis media, often resulting in unnecessary antibiotic use. Hospitalization due to concern for serious infection has been documented in one-third of children less than six months of age with primary infection seen in an ED [7,18]. These data indicate that acute HHV-6B infection

Table 1

Signs and symptoms associated with primary HHV-6 infection.

	Range	Vianna *et al.* [46]	Zerr *et al.* [10]	Caserta *et al.* [43]	Hall *et al.* [7]	Asano *et al.* [17]	Pruksananonda *et al.* [18]
Year published	1992–2008	2008	2005	1998	1994	1994	1992
Number of subjects	626	97	130	29	160 (1094 evaluated)[a]	179	34
Inclusion criteria		Children with rash and/or roseola	Out-patient cohort	Children with fever	Children evaluated in the Emergency Dept.	Children with rash and/or roseola	Children evaluated in the Emergency Dept.
				% of patients with symptom(s) when reported			
Asymptomatic	6		6				
Fever ($T > 38$ °C)	58–98	94	58	100	100 [87 ($T > 39$)]	98	100 [65 ($T > 40$)]
Rash (generalized)	18–91	91	31	48			18
Roseola	17–24		24		17		
Gastrointestinal symptoms (general)	3–34			34	30		3
Vomiting	8–21	21	8				21
Diarrhea	24–68	24	26			68	27
Upper respiratory symptoms	3–41				41		3
Rhinorrhea	56–66	61	66				56
Lower respiratory symptoms	24						24
Cough	27–62	62	34				27
Cervical adenopathy	31–34	34				31	
Pharyngeal papules	65					65	
Tonsillitis	29	29					
Conjunctivitis	26	26					
Acute otitis media/ inflammed tympanic membranes	8–62	8			30		62
Eyelid edema	30					30	
Fussiness/irritability	69–82		70	69			82
Seizures	0–17	1	0	17	13	8	3
Bulging anterior fontanelle	26					26	
Prompted outpatient visit	39		39				
Prompted hospitalization	13–17			17	13		

[a] None of the additional 582 infants with non-febrile illness evaluated in the Emergency Department or the 352 infants without an acute illness seen in ambulatory clinics had evidence of primary HHV-6 infection.

is associated with a high level of healthcare utilization. While the majority of children have a relatively benign clinical course, the acute clinical presentation may be concerning to both parents and healthcare providers alike.

Complications

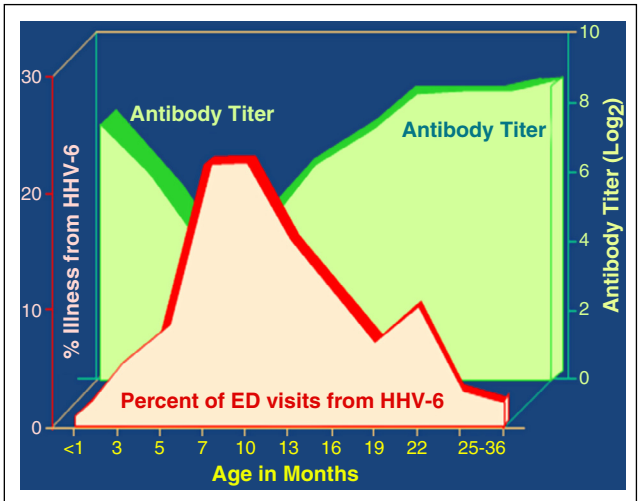
Case reports and small case series have linked primary HHV-6 infection with a wide range of potential complications including myocarditis, rhabdomyolysis, thrombocytopenia, Guillain-Barre syndrome and hepatitis/fulminant hepatic failure [20–22]. Many of these studies used the presence of HHV-6 DNA in the target organ, PBMCs, or other body fluids as evidence of active HHV-6 infection. However, detection of viral nucleic acid can represent active infection, latent infection or ciHHV-6. While such laboratory studies are not widely available, active replication of the virus or protein production should be identified to correlate infection with the clinical syndrome observed. The absence or presence of HHV-6-specific antibody can then be used to help determine whether there is a primary infection or reactivation, respectively.

Neurologic complications and sequelae

Seizures

Neurologic complications, manifested as seizures or encephalopathy, have long been associated with roseola. However, the true prevalence of seizures complicating HHV-6 primary infection has been difficult to determine due to the wide variation in study designs and

Figure 1



HHV-6 antibody titer in 0–36-month-old children and acquisition of primary infection.
Data from Hall CB *et al.*: **Human herpesvirus-6 infection in children: a prospective study of complication and reactivation**. *N Engl J Med* **331**:432–438. Copyright (1994) Massachusetts Medical Society. Reprinted with permission.

populations. A literature review encompassing studies from 1994 to 2004 found that 17% of children seeking medical attention with primary HHV-6 infection had seizures as a complication [23]. Children from 12 to 15 months of age may be at particular risk with a documented rate of febrile seizures of 36% among children presenting to the ED with acute HHV-6B infection. Overall, primary HHV-6B infection accounts for approximately 25–33% of the febrile seizures observed in children less than 24 months of age in the ED setting [7,24]. Additionally, data from the United Kingdom identified HHV-6B & HHV-7 infection in 17% of cases of suspected encephalitis or severe febrile seizures in young children [25].

While the majority of febrile seizures are considered to have a benign clinical course, 5–8% meet criteria for status epilepticus, and an estimated 5000–10 000 cases of febrile status epilepticus (FSE) occur annually in the United States. Febrile seizures are the most common cause of status epilepticus in previously healthy children, accounting for over 70% of status epilepticus during the second year of life [26,27]. The long term consequences of FSE are still not completely understood. There is a potential but controversial link to the future development of intractable temporal lobe epilepsy and hippocampal sclerosis, which is the most common reason for epilepsy surgery in adults [28,29]. Recent data from the multicenter prospective study, Consequences of Prolonged Febrile Seizures in Childhood (FEBSTAT), has substantially expanded our current understanding of FSE [30••]. This study has provided ongoing detailed evaluation of 200 children from ages one month through five years who presented with FSE in order to study the casual relationship between FSE and temporal lobe epilepsy. HHV-6 and HHV-7 virologic studies were performed to determine the frequency of roseolovirus-associated FSE and to determine if roseolovirus-associated FSE is more likely to cause subsequent hippocampal injury and temporal lobe epilepsy. There were 44 cases of primary roseolovirus infection and 14 cases of reactivation as determined by serology and reverse transcriptase PCR. Together, HHV-6B and HHV-7 accounted for one-third of the cases of FSE in the study with HHV-6B causing the majority. There were no differences in acute temporal lobe (hippocampal) injury between children with HHV-6 or HHV-7 infection and those without at the time of infection, and the subsequent development of hippocampal sclerosis is still under active investigation. Therefore, while roseoloviruses may cause hippocampal injury, it appears they may be no more likely than other viruses to do so during the acute illness [30••]. HHV-6B has been found in temporal lobe specimens of patients with intractable temporal lobe epilepsy, but the causal relationship between HHV-6B reactivation and hippocampal injury remains undefined [31–33].

Encephalitis and other neurologic disorders

HHV-6B reactivation is an established cause of limbic encephalitis in immune compromised persons following hematopoetic stem cell transplantation, as initially described by Wainwright and colleagues [34] (*please refer to the accompanying review by Zerr and Hill*). More recently, the receipt of cord blood stem cells has been highly associated with HHV-6 reactivation and encephalitis [35•,36]. HHV-6B, and rarely HHV-7, primary infection has also been associated with encephalitis in immune competent individuals [37]. There appears to be a distinct geographic distribution, with the highest incidence occurring in Japan. Surveys estimate that 60 cases of roseola per year are complicated by encephalitis in Japan, making it the second most common cause of infection-related encephalitis. Severe neurologic sequelae such as acute necrotizing encephalitis, hemorrhagic shock and acute encephalopathy with biphasic seizures complicate nearly half of those cases [38,39]. Evidence suggests that this may be a cytokine-mediated disorder [40]. HHV-6B has also been implicated in triggering potentially fatal neurologic deterioration in children with an underlying mitochondrial disorder involving polymerase gamma gene (POLG) mutations, suggesting that underlying host factors may contribute to the severity of HHV-6-associated neurological disease [41].

HHV-7 primary infection

HHV-7 was first isolated from $CD4^+$ lymphocytes in 1990 by Frenkel and colleagues and subsequently found

to be a distinct virus closely related to HHV6-A and HHV-6B and an additional cause of roseola [42]. Infection is highly prevalent worldwide and also causes universal infection in childhood. However, HHV-7 tends to infect slightly older children when compared to primary HHV-6B infection. A small case series identified 8 cases of primary HHV-7 infection out of 250 children presenting to the ED with fever. The median age of presentation was 26 months and only one child was less than 13 months old. The clinical presentation was indistinguishable from that of HHV-6B infection, and notably six of the eight children presented with seizures [43]. Suga and colleagues in Japan also found that HHV-7 infection was comparable to HHV-6 in a slightly older child, although seizure activity was only observed in one of fifteen cases of HHV-7 primary infection [44]. While these studies are relatively small in size, it appears that HHV-7 primary infection has the potential for severe complications similar to HHV-6. Recent evidence has also linked delayed HHV-7 primary infection with severe neurologic complications, including encephalitis and Guillain–Barre syndrome [45•].

Summary/research priorities

Primary infection with roseoloviruses is nearly universal in early childhood. While the majority of infections are self-limited, the large number of infections coupled with the characteristic fever leads to significant healthcare utilization and possible antibiotic misuse. New methods for sensitive, specific and timely diagnosis of acute infection could potentially mitigate some of the healthcare expenditures and antimicrobial overuse (*please refer to the accompanying review on diagnostics by Hill et al.*). Additionally, the universal nature of infection with roseoloviruses, along with the recognition of ciHHV-6, creates unique challenges in investigating the true burden of disease and research is most urgently needed to determine methodology and criteria for distinguishing a causal relationship between roseoloviruses and pathology. Although primary infection has been directly linked to a spectrum of neurologic complications, most notably febrile status epilepticus, the full spectrum of complications and their clinical burden remain important research questions. The identification of potential biomarkers to predict individuals at high risk for complications and the possible benefits of antiviral treatment in select populations are related research priorities.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M, Halligan G, Biberfeld P, Wong-Staal F, Kramarsky B *et al.*: **Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders**. *Science* 1986, **234**:596-601.

2. Ablashi D, Agut H, Alvarez-Lafuente R, Clark DA, Dewhurst S, DiLuca D, Flamand L, Frenkel N, Gallo R, Gompels UA *et al.*: **Classification of HHV-6A and HHV-6B as distinct viruses**. *Arch Virol* 2014, **159**:863-870.

3. Suga S, Yoshikawa T, Kajita Y, Ozaki T, Asano Y: **Prospective study of persistence and excretion of human herpesvirus-6 in patients with exanthem subitum and their parents**. *Pediatrics* 1998, **102(4 Pt 1)**:900-904.

4. Hall CB, Caserta MT, Schnabel KC, Shelley LM, Carnahan JA, Marino AS, Yoo C, Lofthus GK: **Transplacental congenital human herpesvirus 6 infection caused by maternal chromosomally integrated virus**. *J Infect Dis* 2010, **201**:505-507.

5. Altschuler EL: **Oldest description of roseola and implications for the antiquity of human herpesvirus 6**. *Pediatr Infect Dis J* 2000, **19**:903.

6. Yamanishi K, Okuno T, Shiraki K, Takahashi M, Kondo T, Asano Y, Kurata T: **Identification of human herpesvirus-6 as a causal agent for exanthem subitum**. *Lancet* 1988, **1**:1065-1067.

7. Hall CB, Long CE, Schnabel KC, Caserta MT, McIntyre KM, Costanzo MA, Knott A, Dewhurst S, Insel RA, Epstein LG: **Human herpesvirus-6 infection in children. A prospective study of complications and reactivation**. *N Engl J Med* 1994, **331**:432-438.

8. Yoshikawa T, Suga S, Asano Y, Yazaki T, Kodama H, Ozaki T: **Distribution of antibodies to a causative agent of exanthem subitum (human herpesvirus-6) in healthy individuals**. *Pediatrics* 1989, **84**:675-677.

9. Ward KN, Gray JJ, Fotheringham MW, Sheldon MJ: **IgG antibodies to human herpesvirus-6 in young children: changes in avidity of antibody correlate with time after infection**. *J Med Virol* 1993, **39**:131-138.

10. Zerr DM, Meier AS, Selke SS, Frenkel LM, Huang ML, Wald A, Rhoads MP, Nguy L, Bornemann R, Morrow RA *et al.*: **A population-based study of primary human herpesvirus 6 infection**. *N Engl J Med* 2005, **352**:768-776.

11. Hall CB, Caserta MT, Schnabel KC, Boettrich C, McDermott MP, Lofthus GK, Carnahan JA, Dewhurst S: **Congenital infections with human herpesvirus 6 (HHV6) and human herpesvirus 7 (HHV7)**. *J Pediatr* 2004, **145**:472-477.

12. Bates M, Monze M, Bima H, Kapambwe M, Clark D, Kasolo FC, Gompels UA: **Predominant human herpesvirus 6 variant A infant infections in an HIV-1 endemic region of Sub-Saharan Africa**. *J Med Virol* 2009, **81**:779-789.

13. Rhoads MP, Magaret AS, Zerr DM: **Family saliva sharing behaviors and age of human herpesvirus-6B infection**. *J Infect* 2007, **54**:623-626.

14. Harberts E, Yao K, Wohler JE, Maric D, Ohayon J, Henkin R, Jacobson S: **Human herpesvirus-6 entry into the central nervous system through the olfactory pathway**. *Proc Natl Acad Sci U S A* 2011, **108**:13734-13739.

15. Hall CB, Caserta MT, Schnabel K, Shelley LM, Marino AS, Carnahan JA, Yoo C, Lofthus GK, McDermott MP: **Chromosomal integration of human herpesvirus 6 is the major mode of congenital human herpesvirus 6 infection**. *Pediatrics* 2008, **122**:513-520.

16. • Gravel A, Hall CB, Flamand L: **Sequence analysis of transplacentally acquired human herpesvirus 6 DNA is**

consistent with transmission of a chromosomally integrated reactivated virus. *J Infect Dis* 2013, **207**:1585-1589.
HHV-6 DNA sequence analysis of two mother-infant pairs in which the mothers had ciHHV-6 and infants had transplacentally-acquired HHV-6 suggests that reactivation of maternal ciHHV-6 is a potential mode of congenital HHV-6 infection.

17. Asano Y, Yoshikawa T, Suga S, Kobayashi I, Nakashima T, Yazaki T, Kajita Y, Ozaki T: **Clinical features of infants with primary human herpesvirus 6 infection (exanthem subitum, roseola infantum)**. *Pediatrics* 1994, **93**:104-108.

18. Pruksananonda P, Hall CB, Insel RA, McIntyre K, Pellett PE, Long CE, Schnabel KC, Pincus PH, Stamey FR, Dambaugh TR *et al.*: **Primary human herpesvirus 6 infection in young children**. *N Engl J Med* 1992, **326**:1445-1450.

19. •• Colvin JM, Muenzer JT, Jaffe DM, Smason A, Deych E, Shannon WD, Arens MQ, Buller RS, Lee WM, Weinstock EJ *et al.*: **Detection of viruses in young children with fever without an apparent source**. *Pediatrics* 2012, **130**:e1455-e1462.
Recent prospective study of children less than 3 years of age presenting to ED with undifferentiated fever. HHV-6 DNA was detected in 17% of febrile children, identifying it as a major cause of fever and healthcare utilization in this age group.

20. Fujino M, Ohashi M, Tanaka K, Kato T, Asano Y, Yoshikawa T: **Rhabdomyolysis in an infant with primary human herpesvirus 6 infection**. *Pediatr Infect Dis J* 2012, **31**:1202-1203.

21. Yoshikawa T, Ihira M, Suzuki K, Suga S, Kito H, Iwasaki T, Kurata T, Tanaka T, Saito Y, Asano Y: **Fatal acute myocarditis in an infant with human herpesvirus 6 infection**. *J Clin Pathol* 2001, **54**:792-795.

22. Yoshikawa T, Morooka M, Suga S, Niinomi Y, Kaneko T, Shinoda K, Muraki Y, Takahashi K, Sugaya N, Asano Y: **Five cases of thrombocytopenia induced by primary human herpesvirus 6 infection**. *Acta Paediatr Jpn* 1998, **40**:278-281.

23. Millichap JG, Millichap JJ: **Role of viral infections in the etiology of febrile seizures**. *Pediatr Neurol* 2006, **35**:165-172.

24. Barone SR, Kaplan MH, Krilov LR: **Human herpesvirus-6 infection in children with first febrile seizures**. *J Pediatr* 1995, **127**:95-97.

25. Ward KN, Andrews NJ, Verity CM, Miller E, Ross EM: **Human herpesviruses-6 and -7 each cause significant neurological morbidity in Britain and Ireland**. *Arch Dis Child* 2005, **90**:619-623.

26. Hesdorffer DC, Benn EK, Bagiella E, Nordli D, Pellock J, Hinton V, Shinnar S, Team FS: **Distribution of febrile seizure duration and associations with development**. *Ann Neurol* 2011, **70**:93-100.

27. DeLorenzo RJ, Hauser WA, Towne AR, Boggs JG, Pellock JM, Penberthy L, Garnett L, Fortner CA, Ko D: **A prospective, population-based epidemiologic study of status epilepticus in Richmond, Virginia**. *Neurology* 1996, **46**:1029-1035.

28. Falconer MA, Serafetinides EA, Corsellis JA: **Etiology and pathogenesis of temporal lobe epilepsy**. *Arch Neurol* 1964, **10**:233-248.

29. Shinnar S, Hesdorffer DC, Nordli DR Jr, Pellock JM, O'Dell C, Lewis DV, Frank LM, Moshe SL, Epstein LG, Marmarou A *et al.*: **Phenomenology of prolonged febrile seizures: results of the FEBSTAT study**. *Neurology* 2008, **71**:170-176.

30. •• Epstein LG, Shinnar S, Hesdorffer DC, Nordli DR, Hamidullah A, Benn EK, Pellock JM, Frank LM, Lewis DV, Moshe SL *et al.*: **Human herpesvirus 6 and 7 in febrile status epilepticus: the FEBSTAT study**. *Epilepsia* 2012, **53**:1481-1488.
A multicenter, prospective study of children presenting with febrile status epilepticus associated HHV-6 or HHV-7 infection with one-third of the cases. The possible link to the development of hippocampal injury is under current investigation.

31. Li JM, Lei D, Peng F, Zeng YJ, Li L, Xia ZL, Xia XQ, Zhou D: **Detection of human herpes virus 6B in patients with mesial temporal lobe epilepsy in West China and the possible association with elevated NF-kappaB expression**. *Epilepsy Res* 2011, **94**:1-9.

32. Niehusmann P, Mittelstaedt T, Bien CG, Drexler JF, Grote A, Schoch AJ, Becker S: **Presence of human herpes virus 6 DNA exclusively in temporal lobe epilepsy brain tissue of patients with history of encephalitis**. *Epilepsia* 2010, **51**:2478-2483.

33. Theodore WH, Epstein L, Gaillard WD, Shinnar S, Wainwright MS, Jacobson S: **Human herpes virus 6B: a possible role in epilepsy?** *Epilepsia* 2008, **49**:1828-1837.

34. Wainwright MS, Martin PL, Morse RP, Lacaze M, Provenzale JM, Coleman RE, Morgan MA, Hulette C, Kurtzberg J, Bushnell C *et al.*: **Human herpesvirus 6 limbic encephalitis after stem cell transplantation**. *Ann Neurol* 2001, **50**:612-619.

35. • Hill JA, Koo S, Guzman Suarez BB, Ho VT, Cutler C, Koreth J, Armand P, Alyea EP III, Baden LR, Antin JH *et al.*: **Cord-blood hematopoietic stem cell transplant confers an increased risk for human herpesvirus-6-associated acute limbic encephalitis: a cohort analysis**. *Biol Blood Marrow Transplant* 2012, **18**:1638-1648.
A retrospective cohort study of hematopoietic stem cell transplant recipients identified the receipt of cord blood stem cells as a major risk factor for the development of HHV-6-associated posttransplantation acute limbic encephalitis.

36. Scheurer ME, Pritchett JC, Amirian ES, Zemke NR, Lusso P, Ljungman P: **HHV-6 encephalitis in umbilical cord blood transplantation: a systematic review and meta-analysis**. *Bone Marrow Transplant* 2013, **48**:574-580.

37. Ward KN: **Child and adult forms of human herpesvirus 6 encephalitis: looking back, looking forward**. *Curr Opin Neurol* 2014, **27**:349-355.

38. Yoshikawa T, Ohashi M, Miyake F, Fujita A, Usui C, Sugata K, Suga S, Hashimoto S, Asano Y: **Exanthem subitum-associated encephalitis: nationwide survey in Japan**. *Pediatr Neurol* 2009, **41**:353-358.

39. Hoshino A, Saitoh M, Oka A, Okumura A, Kubota M, Saito Y, Takanashi J, Hirose S, Yamagata T, Yamanouchi H *et al.*: **Epidemiology of acute encephalopathy in Japan, with emphasis on the association of viruses and syndromes**. *Brain Dev* 2012, **34**:337-343.

40. Kawamura Y, Yamazaki Y, Ohashi M, Ihira M, Yoshikawa T: **Cytokine and chemokine responses in the blood and cerebrospinal fluid of patients with human herpesvirus 6B-associated acute encephalopathy with biphasic seizures and late reduced diffusion**. *J Med Virol* 2014, **86**:512-518.

41. Al-Zubeidi D, Pathak TM, Cai S, Schlaggar C, Storch BL, Grange GA, Watson DKME: **Fatal human herpes virus-6 associated encephalitis in two boys with underlying POLG mitochondrial disorders**. *Pediatr Neurol* 2014, **51**:448-452.

42. Frenkel N, Schirmer EC, Wyatt LS, Katsafanas G, Roffman E, Danovich RM, June CH: **Isolation of a new herpesvirus from human CD4$^+$ T cells**. *Proc Natl Acad Sci USA* 1990, **87**:748-752.

43. Caserta MT, Hall CB, Schnabel K, Long CE, D'Heron N: **Primary human herpesvirus 7 infection: a comparison of human herpesvirus 7 and human herpesvirus 6 infections in children**. *J Pediatr* 1998, **133**:386-389.

44. Suga S, Yoshikawa T, Nagai T, Asano Y: **Clinical features and virological findings in children with primary human herpesvirus 7 infection**. *Pediatrics* 1997, **99**:E4.

45. • Schwartz KL, Richardson SE, Ward KN, Donaldson C, MacGregor D, Banwell B, Mahant S, Bitnun A: **Delayed primary HHV-7 infection and neurologic disease**. *Pediatrics* 2014, **133**:e1541-e1547.
Identified three adolescents with severe neurologic disease (encephalitis and Guillaine-Barre syndrome) and evidence of acute HHV-7 primary infection based on CSF PCR and serology. This suggests that delayed acquisition of virus may be linked to severe neurologic complications.

46. Vianna RA, de Oliveira SA, Camacho LA, Knowles W, Brown D, Pereira AC, Velarde LG, Siqueira MM: **Role of human herpesvirus 6 infection in young Brazilian children with rash illnesses**. *Pediatr Infect Dis J* 2008, **27**:533-537.

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Current Opinion in Virology

Past, present, and future perspectives on the diagnosis of Roseolovirus infections

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Diagnosis of Roseolovirus infections mandates careful selection of patients, samples, and testing methods. We review advances in the field and highlight research priorities. Quantitative (q)PCR can accurately identify and distinguish between human herpesvirus 6 (HHV-6) species A and B. Whether screening of high-risk patients improves outcomes is unclear. Chromosomally integrated (ci)HHV-6 confounds test interpretation but can be ruled out with digital PCR. Reverse transcription qPCR may be a more specific and clinically applicable test for actively replicating Roseoloviruses, particularly among patients with ciHHV-6. Interpretation of Roseolovirus test results faces many challenges. However, careful application of refined and emerging diagnostic techniques will allow for increasingly accurate diagnosis of clinically significant infections and disease associations.

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Introduction

The *Roseolovirus* genus of the betaherpesvirus subfamily is composed of three enveloped, double-stranded DNA viruses: human herpesvirus (HHV-) 6A, HHV-6B, and HHV-7 [1]. These viruses share many properties that include virion structure, genomic sequence, and epidemiology but have important molecular and biologic differences [2•]. Like other human herpesviruses, infection with Roseoloviruses occurs early in life, results in chronic viral latency in diverse cell types, and affects the population at large. These characteristics complicate diagnostic efforts to determine whether Roseoloviruses are causative in many implicated diseases. Additional confusion has developed due to the unique ability of HHV-6A and HHV-6B to integrate into chromosomal telomeres of infected cells [3] as reviewed in this issue by Kaufer et al. When this occurs in a germ cell, vertical transmission of inherited chromosomally integrated (ci)HHV-6 results in offspring with latent HHV-6 DNA in every nucleated cell of their body. To further complicate matters, there is evidence that biologically active HHV-6 can reactivate in individuals with inherited ciHHV-6 and cause disease [4,5••,6]. This review highlights important advances in the diagnosis of Roseolovirus infections and provides guidance for application of current and developing diagnostic methods.

Who to test

Roseoloviruses have been variably associated with many diseases in diverse patient groups. Primary HHV-6B infection occurs in the majority of children by two years of age and usually results in a typical presentation of exanthem subitum (roseola) with mild symptoms including fever and rash [7]. HHV-6A and HHV-7 primary infection have epidemiologic differences in comparison to HHV-6B but also appear to occur in childhood with similar presentations [8–10]. Serious complications are infrequent, although primary infection with Roseoloviruses leads to significant healthcare utilization [7], and HHV-6B or HHV-7 have been associated with approximately one-third of cases of febrile status epilepticus [11]. Although testing for Roseoloviruses in the setting of typical exanthem subitum is generally not indicated, quick and accurate diagnosis could play a role in stemming antimicrobial overuse, minimizing unnecessary hospitalization, informing potential utility of selective treatment, and advancing understanding of the clinical impact of primary infection (Table 1). Primary infections are reviewed in detail in this section by Tesini et al.

The majority of known complications due to Roseoloviruses result from HHV-6B reactivation in immunocompromised patients, specifically those undergoing hematopoietic cell (HCT) or solid organ transplantation (SOT) as reviewed in this issue by Hill and Zerr [12]. Selective testing is important among these patients (Table 1). HHV-6B and HHV-7 reactivation after HCT or SOT occurs in 40–50% of patients, whereas HHV-6A reactivation is infrequent [13–15]. HHV-6A and HHV-7 do not appear to be important pathogens in these patients. However, HHV-6B has been associated

Table 1

Summary of key diagnostic considerations for clinical testing of HHV-6B[a]

Patient selection	**Comments**	
• Primary infection	• Rarely results in significant morbidity, routine testing not indicated but may stem inappropriate use of healthcare resources	
• Reactivation after HCT	• Frequent finding with multiple associated complications, targeted testing indicated	
• Other	• Selective testing should be considered in other immunocompromised and immunocompetent patients with HHV-6B-associated complications	
Test selection	**Strengths**	**Weaknesses**
• Quantitative PCR	• Sensitive, quantitative, efficient, distinguishes species	• Not standardized, detects latent virus
• Digital PCR	• Better accuracy and precision, useful for detecting ciHHV-6	• More expensive and labor intensive, detects latent virus
• Reverse transcription PCR	• Positive results represent active replication	• More expensive and labor intensive
Sample selection	**Strengths**	**Weaknesses**
• Whole blood, serum, plasma	• Easy to access and process	• May contain latent virus, not a perfect surrogate for end-organ disease
• Tissue	• Appropriate testing provides stronger evidence for causality	• May contain latent virus, difficult to obtain
• Other (e.g. CSF, BALF)	• Better surrogate for end-organ disease than blood fractions	• May contain latent virus, difficult to obtain

HHV-6, human herpesvirus 6; HCT, hematopoietic cell transplantation; PCR, polymerase chain reaction; ciHHV-6, inherited chromosomally integrated HHV-6; CSF, cerebrospinal fluid; BALF, bronchoalveolar lavage fluid.

[a] Testing for HHV-6A or HHV-7 should be considered on a case-by-case basis, as there is little evidence to support any definitive disease association for either virus.

with many complications in HCT recipients, most notably central nervous system (CNS) disease [13,16,17]. Accordingly, it is reasonable to test transplant recipients for HHV-6B in the setting of any end-organ disease and particularly those with encephalopathy. Although readily available antiviral medications can abrogate viral reactivation when used as a preventive measure, this has not resulted in statistically significant improvement in associated outcomes in a few small studies [18–20]. Whether routine monitoring for HHV-6 in transplant recipients can improve outcomes remains unclear [15].

Testing for Roseoloviruses in other patient groups with findings suggestive of herpesvirus pathogenicity and an otherwise negative workup should be considered (Table 1). Ultimately, testing should be ordered judiciously in all settings, and results must be interpreted in the context of the clinical scenario, sample source, and possibility of inherited ciHHV-6.

Clinical testing and specimen selection

We again underscore that test and specimen selection for Roseolovirus testing should be guided by the clinical context. Direct detection of Roseoloviruses by culture is considered the gold-standard test for active infection, but this method is labor intensive, slow, and unsuitable for routine clinical use [1]. Indirect methods to detect an immunological response have limited utility for clinical use [21]. Numerous serologic assays have been described, including indirect fluorescent-antibody and enzyme-linked immunosorbent assay. IgM testing is not useful for clinical diagnosis of primary infection [22], and most assays are unable to discriminate prior infections with HHV-6A from HHV-6B, although a recently described assay appears to enable variant-specific serologic testing [23]. Current antigenemia tests are inadequate for distinguishing low-level viral reactivation from clinically relevant infection [24,25]. Immunohistochemistry and in situ hybridization are rarely used clinically due to limited sensitivity and slow turn-around time. Selective application of DNA testing by polymerase chain reaction (PCR) assay, however, meets important criteria for clinical use: it is sensitive, quantitative, and precise; it can distinguish between species; and it can be efficiently performed [26•]. Accordingly, PCR for Roseolovirus DNA has become the mainstay of clinical diagnostics. We focus our discussion on diagnostic techniques for HHV-6 species (Table 1).

A variety of qPCR assays for measuring HHV-6 DNA viral load are in clinical use in laboratories across the world [26•,27,28]. Well-validated assays target conserved regions of the HHV-6 genome, and some are able to differentiate HHV-6A and HHV-6B. Early PCR assays that used qualitative, nested approaches had high sensitivity but were prone to false-positive results. Quantitative real-time PCR (qPCR) has emerged as the most sensitive and rapid method available for clinical diagnosis of Roseolovirus infection or reactivation. However, inter-lab quantitative agreement for HHV-6 viral load is poor [27,29], and there is currently no international standard available for HHV-6B or HHV-6A. These factors complicate implementation of commutable assays with clinically meaningful viral load thresholds to validate research findings and guide treatment decisions [30]. The development of an international standard, such as the one for

Table 2

Research priorities

- Study designs that carefully consider patient, diagnostic technique, and sample selection.
- Standardization of Roseolovirus PCR assays and establishment of clinically actionable viral load thresholds.
- Development of optimized RT-qPCR assays for HHV-6B mRNA and correlation with clinically significant HHV-6B-associated diseases.
- Immunologic and tissue-based diagnostics to improve our understanding of the role of Roseoloviruses in associated diseases.

RT-qPCR, reverse transcription real-time polymerase chain reaction.

CMV made available by the World Health Organization [31], would greatly improve inter-lab agreement to better evaluate the association of HHV-6 viral load with associated diseases (Table 2).

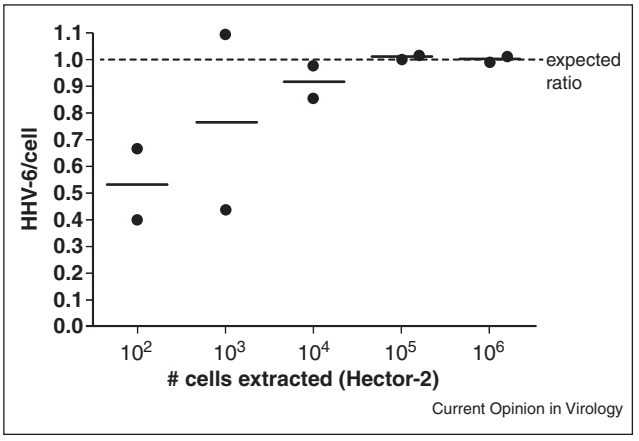
Digital PCR is another method that has recently been utilized for viral quantitation [32•,33•,34] (Table 1). Digital PCR uses the same chemistry as real-time qPCR, but this technique partitions the reaction into thousands of individual droplets, which are each read as positive or negative for DNA template. This allows for absolute quantitation of target DNA without the use of a standard curve [35]. Digital PCR is particularly well suited for the identification of inherited ciHHV-6 [36••,37•]. Previously, ciHHV-6 detection required fluorescence in situ hybridization, a labor-intensive procedure with limited availability, or HHV-6 PCR testing of hair follicle cells [38], an atypical sample type for many molecular diagnostics labs. Although HHV-6 DNA levels of >5.5 $\log_{10}$ copies/ml in whole blood samples is suggestive of inherited ciHHV-6, this can occur in the setting of primary infection or reactivation [3]. A digital PCR assay for inherited ciHHV-6 has been developed to concurrently amplify HHV-6 and human ribonuclease P (RPP30, a reference gene for cell count) DNA; inherited ciHHV-6 is ruled out if the ratio of HHV-6 DNA to cell genome equivalents (two RPP30/cell) falls outside a range of 1 ± 0.07 (Fig. 1) [36••]. This assay has high sensitivity and specificity when used with peripheral blood mononuclear cells (PBMCs) and other cellular samples, but it can also be utilized on study-banked plasma, sera, and other samples to aid in retrospective research, although with reduced specificity. Given mounting evidence to support *in vitro* and *in vivo* HHV-6 reactivation from inherited ciHHV-6 [4,5••,6], adapting this digital PCR method for high-throughput ciHHV-6 screening of immunocompromised individuals at high-risk for HHV-6 reactivation may be important.

Limitations

The use of qPCR to detect Roseolovirus DNA has important limitations (Table 1). Detection of HHV-6 DNA in serum or plasma appears to correlate well with indicators of active replication [39]. This may be misleading in some cases, however, as viral DNA may originate from latently infected cells that have lysed during sample preparation [40]. One study found the specificity of detecting HHV-6 DNA in plasma by qPCR to be 84% compared with viral culture [41]. PCR detection of HHV-6 DNA in plasma or serum is particularly problematic in patients with inherited ciHHV-6 (Fig. 2), who have a high burden of cell-associated latent HHV-6 DNA that can be released, especially if there is a delay in sample preparation and testing [38]. Detection of HHV-6 DNA in whole blood or PBMCs does not correlate as well with active viral replication, as the mononuclear cell is a site of latency [42]. Results of PCR testing of other cellular clinical specimens (e.g. tissue biopsies) can be difficult to interpret for the same reasons.

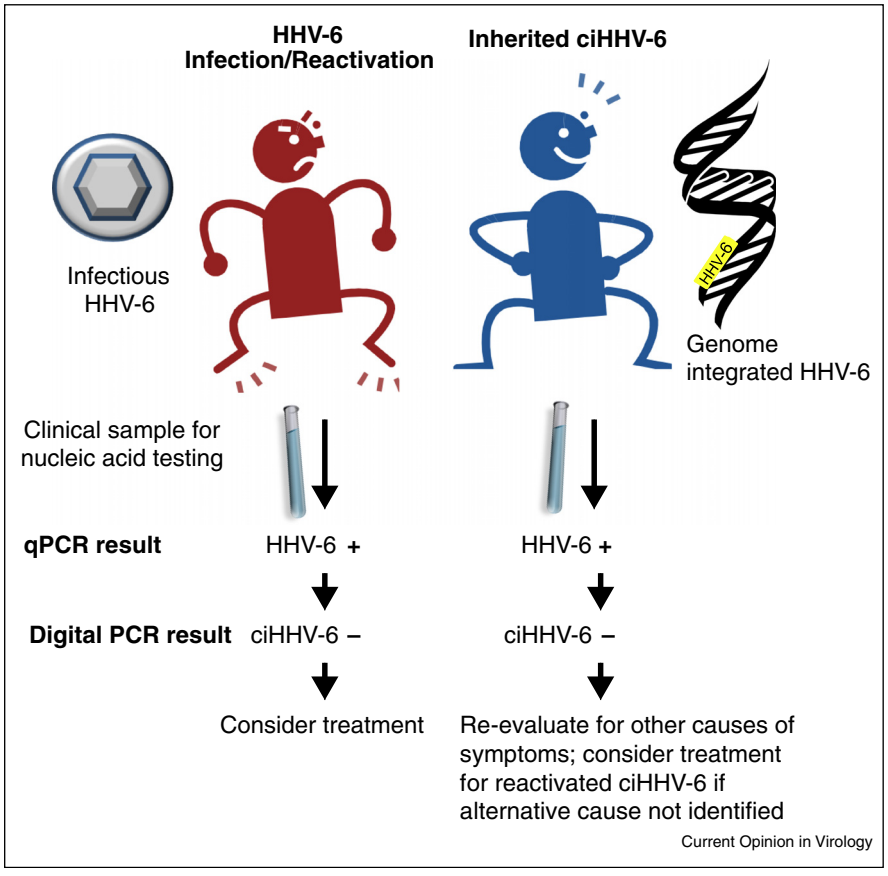
Additional limitations to consider relate to the use of HHV-6 DNA detection in fluid samples (e.g. blood specimens, cerebrospinal fluid [CSF], bronchoalveolar lavage fluid) as a biomarker for end-organ dysfunction (Table 1). Physicians are increasingly reliant on easy-to-access surrogate markers of disease in an effort to minimize invasive procedures, such as a biopsy. However, qPCR for HHV-6 DNA is relatively insensitive for this purpose. Although HHV-6B DNA detection in blood and CSF specimens appears to occur concurrently with most cases of HHV-6B-associated CNS disease, viral detection and viral load thresholds do not strictly predict end-organ disease [43–45]. HHV-6 DNA in CSF and brain samples may also last longer than in blood samples [46,47]. In liver transplant patients with HHV-6-associated graft hepatitis, HHV-6 DNA was infrequently detected in serum [48]. Bronchoalveolar lavage fluid with detectable HHV-6 DNA also appears to be an imperfect surrogate for pulmonary

Fig. 1



Dilution series (10-fold) of Hector-2 ciHHV-6 cell line indicates that the droplet digital PCR assay provides a precise ratio of 1 HHV-6/cell with as few as 10^4 cells. Bars represent the mean of two replicate reactions (denoted by circles).
Source: Reprinted with permission from *Clinical Chemistry*, Vol. 60 no. 5, 765–772.

Fig. 2



Flow diagram of test results and implications in patients with inherited ciHHV-6 versus HHV-6 primary infection or reactivation using quantitative and digital PCR assays for HHV-6 DNA detection.

disease in small studies [49]. Ultimately, PCR for HHV-6 DNA has not provided an ideal means of predicting or diagnosing clinically significant reactivation and pathogenicity. Until a better understanding of risk factors, clinical presentations, and other biomarkers of disease is developed, alternative diagnostic methods that include tissue-based and immunologic studies will be important for defining the role of HHV-6 in associated diseases (Table 2).

Research methods and future directions

While HHV-6 DNA detection with qPCR provides evidence to support active infection, we have reviewed multiple confounding factors that limit the sensitivity of viral DNA detection alone. Research-based methods of culture, serology, immunohistochemistry, and in situ hybridization are useful for identifying active infection and correlating with DNA viral load [50]. However, adaptation of these techniques to routine clinical diagnostics is limited by their complexity, long turn-around time, and variable sensitivity. Perhaps the most promising method for definitive clinical diagnosis of active HHV-6 infection is the molecular detection of viral transcripts via reverse transcription real-time quantitative PCR (RT-qPCR). This method of amplifying messenger (m)RNA from PBMCs or other infected cells could provide a better approach to distinguish active from latent infections [51•], and it may be particularly useful for identifying HHV-6 reactivation in patients with inherited ciHHV-6.

HHV-6 mRNA detection to identify active infection has been reported in a few studies to date. An early study that compared traditional viral culture with a nested RT-PCR assay for the *U100* transcript, expressed during the late stages of viral replication, determined that the RT-PCR assay was 95% sensitive and 98.8% specific for actively replicating virus in PBMC samples [52]. Subsequent studies developed nested RT-PCR assays for genes in other stages of the viral replication cycle, including immediate early genes *U16/17* and *U89/90* [53], early gene *U79/80* [54,55], late gene *U60/66* [53], and latency-associated gene *U94* [56••]. All of these studies were limited by the use of nested RT-PCR, a sensitive but qualitative molecular method historically prone to

false-positive test results. Given these limitations, RT-qPCR assays that effectively quantitate viral transcript levels have been developed [51•,57••]. These assays have targeted immediate early (*U90*), early (*U12*), or late (*U100*) gene transcripts specifically from HHV-6B and show promising results regarding correlation of transcript levels with high-level viremia (>1000 copies/ml DNA) and viral culture in immunocompetent and immunocompromised patients. However, additional steps to optimize findings (e.g. specific processing and storage of clinical samples to augment RNA preservation) are required to further increase sensitivity and standardization. Large studies that correlate transcript detection with DNA detection and active disease will be critical to establish actionable DNA and mRNA transcript thresholds for treatment. Although additional work is needed to validate the utility and feasibility of RT-qPCR in the clinical setting (Table 2), this technique will likely play a bigger role in routine HHV-6 diagnostics, especially in the setting of inherited ciHHV-6.

Conclusions

The definitive establishment of Roseoloviruses as causative pathogens in their many associated diseases is challenging due to the ubiquity of infection, their latency in a variety of cell types, the ability of HHV-6A and HHV-6B to integrate into the human genome, lack of standardized testing metrics, and poor correlation of current diagnostic techniques with end-organ disease. While much work has been done to advance our understanding of the molecular virology, pathogenesis, and disease associations of these viruses, additional studies using immunologic and tissue-based diagnostics will be important to establish the role of Roseoloviruses in end-organ disease and inform clinically applicable testing methods. Ultimately, Roseolovirus detection does not necessarily imply causation, and interpretation of test results must account for the clinical context, sample type, and diagnostic technique in order to formulate valid clinical and scientific conclusions.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Yamanishi K, Mori Y, Pellett PE: **Human herpesvirus 6 and 7**. In *In fields virology*. Edited by Knipe D, Howley P. Wolters Kluwer Health; 2013:2058.

2. • Ablashi D, Agut H, Alvarez-Lafuente R, Clark DA, Dewhurst S, Diluca D, Flamand L, Frenkel N, Gallo R, Gompels UA *et al.*: **Classification of HHV-6A and HHV-6B as distinct viruses**. *Arch Virol* 2014, **159**:863-870.

A review of the distinctions between HHV-6A and HHV-6B to highlight the importance of diagnostic differentiation of these viruses.

3. Pellett P, Ablashi D: **Chromosomally integrated human herpesvirus 6: questions and answers**. *Rev Med Virol* 2012 http://dx.doi.org/10.1002/rmv.

4. Arbuckle JH, Medveczky MM, Luka J, Hadley SH, Luegmayr A, Ablashi D, Lund TC, Tolar J, De Meirleir K, Montoya JG *et al.*: **The latent human herpesvirus-6A genome specifically integrates in telomeres of human chromosomes in vivo and in vitro**. *Proc Natl Acad Sci USA* 2010, **107**:5563-5568.

5. •• Endo A, Watanabe K, Ohye T, Suzuki K, Matsubara T, Shimizu N, Kurahashi H, Yoshikawa T, Katano H, Inoue N *et al.*: **Molecular and virological evidence of viral activation from chromosomally integrated HHV-6A in a patient with X-SCID**. *Clin Infect Dis* 2014 http://dx.doi.org/10.1093/cid/ciu323.

A study utilizing multiple diagnostic methodologies to demonstrate HHV-6 reactivation from a patient with inherited ciHHV-6.

6. Flamand L: **Pathogenesis from the reactivation of chromosomally-integrated HHV-6: Facts rather than fiction**. *Clin Infect Dis* 2014 http://dx.doi.org/10.1093/cid/ciu326.

7. Zerr DM, Meier AS, Selke SS, Frenkel LM, Huang M-L, Wald A, Rhoads MP, Nguy L, Bornemann R, Morrow RA *et al.*: **A population-based study of primary human herpesvirus 6 infection**. *N Engl J Med* 2005, **352**:768-776.

8. De Bolle L, Naesens L, De Clercq E: **Update on human herpesvirus 6 biology, clinical features, and therapy**. *Clin Microbiol Rev* 2005, **18**:217-245.

9. Bates M, Monze M, Bima H, Kapambwe M, Clark D, Kasolo FC, Gompels UA: **Predominant human herpesvirus 6 variant A infant infections in an HIV-1 endemic region of Sub-Saharan Africa**. *J Med Virol* 2009, **81**:779-789.

10. Hall CB, Caserta MT, Schnabel KC, McDermott MP, Lofthus GK, Carnahan JA, Gilbert LM, Dewhurst S: **Characteristics and acquisition of human herpesvirus (HHV) 7 infections in relation to infection with HHV-6**. *J Infect Dis* 2006, **193**:1063-1069.

11. Epstein LG, Shinnar S, Hesdorffer DC, Nordli DR, Hamidullah A, Benn EKT, Pellock JM, Frank LM, Lewis DV, Moshe SL *et al.*: **Human herpesvirus 6 and 7 in febrile status epilepticus: the FEBSTAT study**. *Epilepsia* 2012, **53**:1481-1488.

12. Hill JA, Zerr DM: **Roseoloviruses in transplant recipients: clinical consequences and prospects for treatment and prevention trials**. *Curr Opin Virol* 2014.

13. Zerr DM, Boeckh M, Delaney C, Martin PJ, Xie H, Adler AL, Huang M-L, Corey L, Leisenring WM: **HHV-6 reactivation and associated sequelae after hematopoietic cell transplant**. *Biol Blood Marrow Transplant* 2012, **18**:1700-1708.

14. Razonable RR: **Human herpesviruses 6, 7 and 8 in solid organ transplant recipients**. *Am J Transplant* 2013, **13**:67-78.

15. Olson AL, Dahi PB, Zheng J, Devlin SM, Lubin M, Gonzales AM, Giralt SA, Perales M-A, Papadopoulos EB, Ponce DM *et al.*: **Frequent human herpesvirus-6 viremia but low incidence of encephalitis in double-unit cord blood recipients transplanted without antithymocyte globulin**. *Biol Blood Marrow Transplant* 2014 http://dx.doi.org/10.1016/j.bbmt.2014.02.010.

16. Ogata M, Satou T, Kadota J-I, Saito N, Yoshida T, Okumura H, Ueki T, Nagafuji K, Kako S, Uoshima N *et al.*: **Human herpesvirus 6 (HHV-6) reactivation and HHV-6 encephalitis after allogeneic hematopoietic cell transplantation: a multicenter, prospective study**. *Clin Infect Dis* 2013, **57**:671-681.

17. Dulery R, Salleron J, Dewilde A, Rossignol J, Boyle EM, Gay J, de Berranger E, Coiteux V, Jouet J-P, Duhamel A *et al.*: **Early human herpesvirus type 6 reactivation after allogeneic stem cell transplantation: a large-scale clinical study**. *Biol Blood Marrow Transplant* 2012, **18**:1080-1089.

18. Galarraga MC, Gomez E, de Oña M, Rodriguez A, Laures A, Boga JA, Melon S: **Influence of ganciclovir prophylaxis on citomegalovirus, human herpesvirus 6, and human herpesvirus 7 viremia in renal transplant recipients**. *Transplant Proc* 2005, **37**:2124-2126.

19. Ogata M, Satou T, Inoue Y, Takano K, Ikebe T, Ando T, Ikewaki J, Kohno K, Nishida A, Saburi M *et al.*: **Foscarnet against human herpesvirus (HHV)-6 reactivation after allo-SCT: breakthrough HHV-6 encephalitis following antiviral prophylaxis**. *Bone Marrow Transplant* 2012, **48**:257-264.

20. Ishiyama K, Katagiri T, Hoshino T, Yoshida T, Yamaguchi M, Nakao S: **Preemptive therapy of human herpesvirus-6 encephalitis with foscarnet sodium for high-risk patients after hematopoietic SCT**. *Bone Marrow Transplant* 2011, **46**:863-869.

21. Ward KN: **The natural history and laboratory diagnosis of human herpesviruses-6 and -7 infections in the immunocompetent**. *J Clin Virol* 2005, **32**:183-193.

22. De Oliveira Vianna RA, Siqueira MM, Camacho LAB, Setúbal S, Knowles W, Brown DW, de Oliveira SA: **The accuracy of anti-human herpesvirus 6 IgM detection in children with recent primary infection**. *J Virol Methods* 2008, **153**:273-275.

23. Thäder-Voigt A, Jacobs E, Lehmann W, Bandt D: **Development of a microwell adapted immunoblot system with recombinant antigens for distinguishing human herpesvirus (HHV)6A and HHV6B and detection of human cytomegalovirus**. *Clin Chem Lab Med* 2011, **49**:1891-1898.

24. Volin L, Lautenschlager I, Juvonen E, Nihtinen A, Anttila V-J, Ruutu T: **Human herpesvirus 6 antigenaemia in allogeneic stem cell transplant recipients: impact on clinical course and association with other beta-herpesviruses**. *Br J Haematol* 2004, **126**:690-696.

25. Sampaio AM, Thomasini RL, Guardia AC, Stucchi RSB, Rossi CL, Costa SCB, Boin IFSF: **Cytomegalovirus, human herpesvirus-6, and human herpesvirus-7 in adult liver transplant recipients: diagnosis based on antigenemia**. *Transplant Proc* 2011, **43**:1357-1359.

26. • Cassina G, Russo D, De Battista D, Broccolo F, Lusso P, Malnati MS: **Calibrated real-time polymerase chain reaction for specific quantitation of HHV-6A and HHV-6B in clinical samples**. *J Virol Methods* 2013, **189**:172-179.
A description of the development of two sensitive qPCR assays for HHV-6A and HHV-6B DNA detection in clinical specimens.

27. Flamand L, Gravel A, Boutolleau D, Alvarez-Lafuente R, Jacobson S, Malnati MS, Kohn D, Tang Y-W, Yoshikawa T, Ablashi D: **Multicenter comparison of PCR assays for detection of human herpesvirus 6 DNA in serum**. *J Clin Microbiol* 2008, **46**:2700-2706.

28. Karlsson T, Mannonen L, Loginov R, Lappalainen M, Höckerstedt K, Lautenschlager I: **Development of a new quantitative real-time HHV-6-PCR and monitoring of HHV-6 DNAaemia after liver transplantation**. *J Virol Methods* 2012, **181**:25-36.

29. De Pagter PJ, Schuurman R, de Vos NM, Mackay W, van Loon AM: **Multicenter external quality assessment of molecular methods for detection of human herpesvirus 6**. *J Clin Microbiol* 2010, **48**:2536-2540.

30. Agut H: **Deciphering the clinical impact of acute human herpesvirus 6 (HHV-6) infections**. *J Clin Virol* 2011, **52**:164-171.

31. Hirsch HH, Lautenschlager I, Pinsky BA, Cardeñoso L, Aslam S, Cobb B, Vilchez RA, Valsamakis A: **An international multicenter performance analysis of cytomegalovirus load tests**. *Clin Infect Dis* 2013, **56**:367-373.

32. • Hayden RT, Gu Z, Ingersoll J, Abdul-Ali D, Shi L, Pounds S, Caliendo AM: **Comparison of droplet digital pcr to real-time pcr for quantitative detection of cytomegalovirus**. *J Clin Microbiol* 2012 http://dx.doi.org/10.1128/JCM.02620-12.
A study detailing the direct comparison between qPCR and digital PCR in viral diagnostics.

33. • Sedlak RH, Cook L, Cheng A, Magaret A, Jerome KR: **Clinical utility of droplet digital PCR for human cytomegalovirus**. *J Clin Microbiol* 2014, **52**:2844-2848.
A review of the advantages of digital PCR for clinical virology diagnostics.

34. Hall Sedlak R, Jerome KR: **The potential advantages of digital PCR for clinical virology diagnostics**. *Expert Rev Mol Diagn* 2014, **14**:501-507.

35. Hindson BJ, Ness KD, Masquelier DA, Belgrader P, Heredia NJ, Makarewicz AJ, Bright IJ, Lucero MY, Hiddessen AL, Legler TC *et al.*: **High-throughput droplet digital PCR system for absolute quantitation of DNA copy number**. *Anal Chem* 2011, **83**:8604-8610.

36. •• Sedlak RH, Cook L, Huang M-L, Magaret A, Zerr DM, Boeckh M, Jerome KR: **Identification of chromosomally integrated human herpesvirus 6 by droplet digital PCR**. *Clin Chem* 2014, **60**:765-772.
A study of the first clinically validated viral assay using digital PCR to identify patients with inherited ciHHV-6.

37. • Leibovitch EC, Brunetto GS, Caruso B, Fenton K, Ohayon J, Reich DS, Jacobson S: **Coinfection of human herpesviruses 6A (HHV-6A) and HHV-6B as demonstrated by novel digital droplet PCR assay**. *PLOS ONE* 2014, **9**:e92328.
A study of the use of digital PCR to identify HHV-6A and HHV-6B infection, as well as inherited ciHHV-6, in clinical samples from patients with multiple sclerosis.

38. Ward K, Leong H: **Human herpesvirus 6 chromosomal integration in immunocompetent patients results in high levels of viral DNA in blood, sera, and hair follicles**. *J Clin Microbiol* 2006, **44**:1571-1574.

39. Boutolleau D, Fernandez C, André E, Imbert-Marcille B-M, Milpied N, Agut H, Gautheret-Dejean A: **Human herpesvirus (HHV)-6 and HHV-7: two closely related viruses with different infection profiles in stem cell transplantation recipients**. *J Infect Dis* 2003, **187**:179-186.

40. Achour A, Boutolleau D, Slim A, Agut H, Gautheret-Dejean A: **Human herpesvirus-6 (HHV-6) DNA in plasma reflects the presence of infected blood cells rather than circulating viral particles**. *J Clin Virol* 2007, **38**:280-285.

41. Caserta MT, Hall CB, Schnabel K, Lofthus G, Marino A, Shelley L, Yoo C, Carnahan J, Anderson L, Wang H: **Diagnostic assays for active infection with human herpesvirus 6 (HHV-6)**. *J Clin Virol* 2010, **48**:55-57.

42. Kondo K, Kondo T, Okuno T, Takahashi M, Yamanishi K: **Latent human herpesvirus 6 infection of human monocytes/macrophages**. *J Gen Virol* 1991, **72(Pt 6)**:1401-1408.

43. Hill JA, Boeckh MJ, Sedlak RH, Jerome KR, Zerr DM: **Human herpesvirus 6 can be detected in cerebrospinal fluid without associated symptoms after allogeneic hematopoietic cell transplantation**. *J Clin Virol* 2014 http://dx.doi.org/10.1016/j.jcv.2014.07.001.

44. Hill JA, Koo S, Guzman Suarez BB, Ho VT, Cutler C, Koreth J, Armand P, Alyea EP, Baden LR, Antin JH *et al.*: **Cord-blood hematopoietic stem cell transplant confers an increased risk for human herpesvirus-6-associated acute limbic encephalitis: a cohort analysis**. *Biol Blood Marrow Transplant* 2012, **18**:1638-1648.

45. Ogata M, Kikuchi H, Satou T, Kawano R, Ikewaki J, Kohno K, Kashima K, Ohtsuka E, Kadota J-I: **Human herpesvirus 6 DNA in plasma after allogeneic stem cell transplantation: incidence and clinical significance**. *J Infect Dis* 2006, **193**:68-79.

46. Fotheringham J, Akhyani N, Vortmeyer A, Donati D, Williams E, Oh U, Bishop M, Barrett J, Gea-Banacloche J, Jacobson S: **Detection of active human herpesvirus-6 infection in the brain: correlation with polymerase chain reaction detection in cerebrospinal fluid**. *J Infect Dis* 2007, **195**:450-454.

47. Zerr DM, Gupta D, Huang M-L, Carter R, Corey L: **Effect of antivirals on human herpesvirus 6 replication in hematopoietic stem cell transplant recipients**. *Clin Infect Dis* 2002, **34**:309-317.

48. Pischke S, Gösling J, Engelmann I, Schlue J, Wölk B, Jäckel E, Meyer-Heithuis C, Lehmann U, Strassburg CP, Barg-Hock H *et al.*: **High intrahepatic HHV-6 virus loads but neither CMV nor EBV are associated with decreased graft survival after diagnosis of graft hepatitis**. *J Hepatol* 2012, **56**:1063-1069.

49. Nagate *et al.*: **Detection and quantification of human herpesvirus 6 genomes using bronchoalveolar lavage fluid in immunocompromised patients with interstitial pneumonia**. *Int J Mol Med* 2001, **8**:379.

50. Crawford JR, Santi MR, Thorarinsdottir HK, Cornelison R, Rushing EJ, Zhang H, Yao K, Jacobson S, Macdonald TJ: **Detection of human herpesvirus-6 variants in pediatric brain tumors: association of viral antigen in low grade gliomas**. *J Clin Virol* 2009, **46**:37-42.

51. • Ihira M, Enomoto Y, Kawamura Y, Nakai H, Sugata K, Asano Y, Tsuzuki M, Emi N, Goto T, Miyamura K *et al.*: **Development of quantitative RT-PCR assays for detection of three classes of HHV-6B gene transcripts**. *J Med Virol* 2012, **84**:1388-1395.
A study demonstrating the utility of a RT-qPCR assay for identifying active HHV-6B infection in patients with primary infection and reactivation after HCT.

52. Norton RA, Caserta MT, Hall CB, Schnabel K, Hocknell P, Dewhurst S: **Detection of human herpesvirus 6 by reverse transcription-PCR**. *J Clin Microbiol* 1999, **37**:3672-3675.

53. Van den Bosch G, Locatelli G, Geerts L, Fagà G, Ieven M, Goossens H, Bottiger D, Oberg B, Lusso P, Berneman ZN: **Development of reverse transcriptase PCR assays for detection of active human herpesvirus 6 infection**. *J Clin Microbiol* 2001, **39**:2308-2310.

54. Kondo K, Kondo T, Shimada K, Amo K, Miyagawa H, Yamanishi K: **Strong interaction between human herpesvirus 6 and peripheral blood monocytes/macrophages during acute infection**. *J Med Virol* 2002, **67**:364-369.

55. Pradeau K, Bordessoule D, Szelag J-C, Rolle F, Ferrat P, Le Meur Y, Turlure P, Denis F, Ranger-Rogez S: **A reverse transcription-nested PCR assay for HHV-6 mRNA early transcript detection after transplantation**. *J Virol Methods* 2006, **134**:41-47.

56. •• Strenger V, Caselli E, Lautenschlager I, Schwinger W, Aberle SW, Loginov R, Gentili V, Nacheva E, DiLuca D, Urban C: **Detection of HHV-6-specific mRNA and antigens in PBMCs of individuals with chromosomally integrated HHV-6 (ciHHV-6)**. *Clin Microbiol Infect* 2014 http://dx.doi.org/10.1111/1469-0691.12639.
A study using RT-PCR to demonstrate viral gene expression in four of eleven patients with inherited ciHHV-6.

57. •• Bressollette-Bodin C, Nguyen TVH, Illiaquer M, Besse B, Peltier C, Chevallier P, Imbert-Marcille B-M: **Quantification of two viral transcripts by real time PCR to investigate human herpesvirus type 6 active infection**. *J Clin Virol* 2014, **59**:94-99.
A study of the utility of RT-qPCR for monitoring HHV-6 transcription in HCT recipients.

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Current Opinion in Virology

Roseomics: a blank slate

Nathaniel J Moorman[1] and Eain A Murphy[2]

Recent technological advances have led to an explosion in the system-wide profiling of biological processes in the study of herpesvirus biology, herein referred to as '-omics'. In many cases these approaches have revealed novel virus-induced changes to host cell biology that can be targeted with new antiviral therapeutics. Despite these successes, -omics approaches are not widely applied in the study of roseoloviruses. Here we describe examples of how -omics studies have shaped our understanding of herpesvirus biology, and discuss how these approaches might be used to identify host and viral factors that mediate roseolovirus pathogenesis.

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Introduction

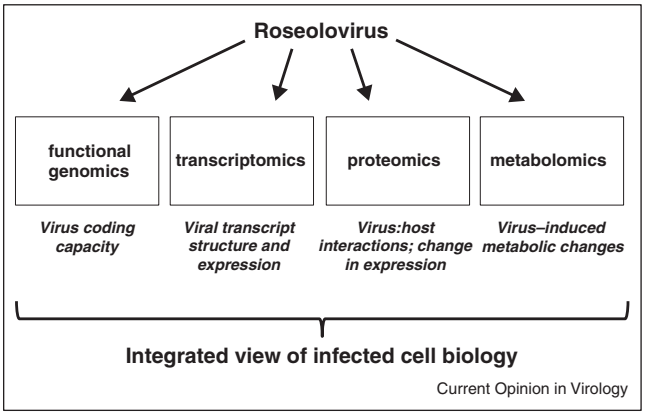
Over the past several years, advancements in high-throughput systems biology approaches and technologies have resulted in major vertical achievements in the field of molecular biology. For the purpose of this review, omics refers to a systems biology approach for defining the components of a biological system and their interactions with one another. Common examples of -omics approaches include (but are not limited to) functional genomics, transcriptomics, metabolomics, and proteomics (reviewed in [1]). These approaches have been successfully applied to several herpesviruses including human herpesvirus 8 (HHV8; also known as Kaposi's sarcoma-associated herpesvirus or KSHV), herpes simplex virus (HSV), human cytomegalovirus (HCMV) and murid herpesvirus 4 (MuHV4; also known as murine gammaherpesvirus-68 or MHV-68) which are discussed below. In each case these studies have vastly expanded our understanding of the unique biology of herpesvirus-infected cells. The unbiased nature of -omics studies has led to a wealth of innovative, testable hypotheses and identified novel virus-induced changes to host signaling pathways that impact viral replication and subsequent pathogenesis. Although genomic and transcriptomic studies have identified new herpesvirus coding regions and transcripts, metabolomics and proteomics studies have defined novel interactions between viruses and host metabolic pathways and protein complexes. Although each of these approaches alone provides a wealth of information, integrating the various -omics approaches generates a holistic understanding of the host–pathogen interactome (Figure 1).

By contrast to the other herpesviruses, few if any -omics approaches have been applied to the study roseoloviruses, comprised of human herpesviruses 6A, 6B and 7. It is now feasible to apply most, if not all, of the standard -omics approaches to the study of roseoloviruses. In this review we highlight opportunities for -omics approaches to rapidly advance our understanding of rosoleovirus biology and describe crucial unmet research needs where -omics approaches should prove useful.

Functional genomics

Functional genomics encompasses the fields of genome sequencing, comparative analysis of related genomes, and screening of phenotypic changes within an organism upon disruption of a candidate open reading frame (ORF). Within a decade after the first roseolovirus genome was discovered in 1986 [2], representative genomes of HHV-6A, HHV-6B and HHV-7 were sequenced (Accession #s NC_001664, AF_157706 and U43400 respectively). Since the original sequencing of these viruses, additional full-length genomes were reported [3–7]. Due to distinct characteristics of these viruses including cellular and tissue tropism, genomic arrangement, peptide coding capacity and subsequent pathogenesis [8,9], each of these roseoloviruses are classified as separate viruses [10]. These studies were the first examples of an -omics approach in the study of roseoloviruses. *In silico* analyses revealed roseolovirus coding regions conserved across herpesvirus families, conserved amongst roseoloviruses, and most importantly, those unique to each roseolovirus [3–5]. For many roseolovirus genes, putative functions were inferred based on homology to orthologous herpesvirus genes of known function. In addition these analyses allowed for focus on novel ORFs that may be involved in distinct roseolovirus pathogenesis. These studies provided the first insights into the organization and potential functional capacity of roseoloviruses.

Figure 1



Schematic representation of high throughput system-wide analyses of roseolovirus pathogenic potential. The above is a listing of several available '-omics' analyses that are suited for profiling the impact of roseolovirus infection on host cells.

Subsequent functional genomics studies have predominantly employed reverse genetics approaches, identifying phenotypic changes resulting from mutation or deletion of a specific viral ORF. These studies have almost exclusively focused on HHV-6A, as it is the only roseolovirus genome that has been cloned into a bacterial artificial chromosome (BAC) [11] and is therefore applicable to genetic manipulation. Examples of reverse genetic approaches in roseoloviruses include defining the role of viral glycoproteins in replication [11], and confirming the role of homologous genes conserved across the herpesvirus family [12].

Several additional functional genomics approaches have been successfully used in other herpesviruses and are amenable to the study of roseoloviruses. Although only a handful of roseolovirus genomes have been sequenced, multiple full-length genome sequences are available for many other human herpesviruses. Comparison of the coding capacity of multiple strains has led to a better understanding of both virus evolution and replication [13]. An excellent example is HCMV, where clinical strains were found to contain approximately 15 kb of DNA that is deleted or inverted during laboratory passage thereby altering the growth and tropism of these strains [14]. Only upon additional sequencing of clinical isolates was it found that these alterations resulted in the loss-of-function of cytomeglaovirus genes essential for viral latency [15]. Presumably roseoloviruses display a similar degree of heterogeneity, however this hypothesis has not been tested. The widespread availability of next generation sequencing coupled with advances in genome analysis and assembly makes this an attractive area for future roseolovirus studies.

Forward genetics approaches have also proven useful in defining novel functions for herpesvirus genes. Forward genetics refers to the process of screening random mutants for a specified phenotype. Subsequent genotyping of the selected variants then reveals the underlying gene (or genes) controlling the phenotype. Many herpesviruses including HCMV, HSV1, and MuHV4 have used genome-wide forward genetics studies to characterize viral genes. Libraries of expression vectors containing herpesvirus ORFs have proven useful in forward genetic screens to identify novel functions for viral genes, including antagonism of the host antiviral response [16] and manipulation of the cell cycle [17]. In addition, global mutagenesis approaches have identified genes in MuHV4 [18], MCMV [19] and HCMV [20,21] needed for efficient virus replication. A global determination of the complement of HHV-6A coding regions required for replication should be employed to identify novel targets for antiviral drugs. Similarly, a comprehensive collection of expression vectors for roseolovirus genes would allow for the identification of viral genes that contribute to unique aspects of the roseolovirus life cycle, for example genome integration. Although HHV-6A, -6B and HHV-7 genomes have each been annotated, the field is hampered by a lack of BAC constructs for HHV-6B and HHV-7, thus making the essential development of forward genetics screens currently unavailable.

Transcriptomics

The most common, and often first employed, analysis of viral infection is monitoring changes in both viral and cellular transcription. High throughput qualitative profiling of transcript changes often relies on microarray technologies where one can monitor literally thousands of cellular transcripts or complete annotated viral transcripts in a single experiment. This technology has been used successfully to study the kinetics of both HHV-6A and HHV-6B transcription [22,23]. This powerful resource is important for identifying the timing and relative levels of transcription from the viral genome. However, this methodology lacks absolute quantification and is biased toward regions of the genome that are previously known to be transcribed, as predefined probe sets are used as bait for transcripts. Next generation sequencing approaches can be used to address these deficiencies. These approaches allow for the unbiased profiling of both cellular and viral transcripts at saturating levels, thereby providing insight into both the absolute levels of transcripts and also transcript structure (reviewed in [24]). Although RNA based deep sequencing has identified roseolovirus transcripts in a few patient samples [25,26], and RNA based deep sequencing has been used to identify small non-coding RNAs encoded by HHV-6B [27], a targeted approach to define roseolovirus mRNAs in a controlled infection has surprisingly not yet been performed. Such an approach would likely refine our understanding of the

temporal expression of rosoleovirus genes, and potentially identify novel coding regions of the viral genome.

Alternative approaches to next generation RNA sequencing have been used with success for other herpesviruses including reverse transcriptase coupled real time quantitative PCR (RT-qPCR) whole genome panels (e.g. [28]) as well as the use of tiled arrays (e.g. [29]). The use of a tiled array for the related herpesvirus MuHV4 allowed for highly reproducible quantitative and qualitative resolution (20nt) of viral transcription during both lytic replication and reactivation from latency [29]. In this study, not only were the kinetics of viral transcription assessed but the authors identified a previously undefined ORF that was not characterized by *in silico* analysis, underscoring the need for multiple -omics approaches for roseolovirus studies. Each of these above mentioned technologies are readily applicable and necessary for understanding the viral lifecycle of roseoloviruses and thus should be prioritized.

Proteomics

Proteomics concerns the large-scale study of structure, modification, function and abundance of proteins. Once a rarity, proteomics approaches have become increasingly common in the study of herpesviruses, specifically in defining viral protein function. Many herpesvirus proteins bear little or no homology to cellular proteins, limiting the ability to infer functional roles based on amino acid sequence conservation. However defining the interacting partners for viral proteins in the context of infection is an effective means for identifying potential functional roles. Typically this approach involves generating a virus strain in which the protein of interest is fused to an epitope tag. The 'tagged' viral protein is then affinity purified from infected cells lysates, and the associated host and/or viral proteins are identified by mass spectrometry [30]. This approach has been used successfully to define novel functions for numerous herpesvirus proteins (e.g. [31–33]), although it has yet to be extended to the study of roseolovirus protein functions.

A related approach can be used to identify changes in post-translation modifications (PTMs) of host and viral proteins during infection. Antibodies to a specific PTM are used as an affinity reagent, and the resulting immune complexes are analyzed by mass spectrometry. This approach is especially useful in defining changes in signaling pathways caused by infection. For example, phospho-proteomic analysis of MuHV4 infected cells identified virus-induced changes to multiple cellular signaling pathways [34], several of which are important for efficient virus replication. These approaches are easily extendable to the study of HHV6A, as a genetically tractable BAC clone exists as well as an efficient *in vitro* lytic replication model.

Quantitative whole cell proteomics (qWCP) is another approach used to identify changes in host protein expression during viral infection. qWCP approaches most commonly use a mass spectrometer to identify either relative or absolute quantities of proteins in cell lysates under different conditions. Both relative and absolute qWCP approaches are standardized, and we direct the reader to several method and review articles for additional details [35,36]. A modification of qWCP has also been developed that defines changes in cell surface proteins during infection [37]. More recently whole cell proteomics coupled with transcript analysis has been used to identify and quantify the temporal expression of known and novel viral proteins [38]. These studies have revealed that herpesvirus proteomes are far more complex than previously appreciated. Given the impact of proteomics on our understanding of herpesvirus protein function and genome complexity, applying these approaches to the study of roseoloviruses should rapidly increase our understanding of these complicated pathogens.

Metabolomics

Another -omics approach applied to the study of herpesvirus biology is metabolomics. The goal of metabolomics is to measure the abundance of all metabolites in a cell. Viruses are obligate intercellular pathogens that are directly reliant on host cell metabolites for anabolic processes; therefore herpesviruses must manipulate metabolic processes to support virus replication. Most metabolomics approaches utilize mass spectrometry to quantify a large number of metabolites from a single sample (reviewed in [39]). Comparing the abundance of specific metabolites in virus-infected cells to that of mock-infected cells reveals crucial virus-induced metabolic changes. Alternatively virus-induced changes to the rate of metabolism can be quantified by measuring the conversion of isotopically-labeled precursor metabolites such as glucose or glutamine into downstream metabolites.

Both approaches have been used to characterize how herpesvirus infection modulates metabolism. For example, both HHV-8 and HCMV increase aerobic glycolysis and stimulate fatty acid synthesis, reminiscent of the metabolic changes observed during oncogenesis [40–42]. By contrast, HSV1 preferentially increases glucose metabolism by the pentose phosphate pathway, presumably to generate sufficient nucleotides for viral DNA replication [43]. Fatty acid synthesis inhibitors limit HCMV replication [44] and the growth of primary effusion lymphomas associated with HHV-8 infection [45], and inhibitors of nucleotide metabolism decrease HSV1 replication [46]. Therefore it is proposed that virus-induced changes in metabolism are promising targets for new antiviral therapeutics [47].

Based on the crucial role for metabolic remodeling in the lytic replication cycle of other herpesviruses, we hypothesize that roseoleviruses modulate host metabolic

pathways in similar yet distinct ways to support efficient virus replication. Uncovering mechanisms of virus-induced metabolic remodeling will likely result in new targeted therapeutic interventions directed at roseoloviruses. Unfortunately almost nothing is known of the effects of roseolovirus infection on host cell metabolism. As such, current drugs used to treat roseolovirus infections are based solely on inferred functions putatively shared with other herpesviruses [48]. Although defining new viral functions to target with novel antivirals will likely require much effort, virus-induced changes in metabolism should be relatively straightforward to define. Therefore metabolomics analyses of roseolovirus infections present a promising direction in the identification of new therapeutics to limit roseolovirus pathogenesis.

Unmet needs

Although we have described different -omics applications that are accessible to roseolovirus researchers, we, as a field, lack several resources needed to advance our understanding of roseolovirus biology to the level of its herpesvirus cousins. For HHV-6B and HHV-7, the lack of an infectious BAC clone is perhaps the most significant barrier to -omics approaches. The absence of HHV-6B and HHV-7 BAC clones severely limits our ability to perform both forward and reverse genetic screens to define the complement of viral factors important for roseolovirus disease. The BACs would also provide a convenient starting point to generate a library of expression vectors for roseolovirus ORFs. This library would be a high value resource for forward genetics screens, such as screens to identify viral proteins that regulate the innate and adaptive immune response. This is arguably the most significant roadblock to roseolovirus research.

We also lack a sufficient understanding of genetic diversity amongst roseoloviruses. A functional genomics analysis of circulating roseolovirus strains in distinct patient populations or locations would likely prove invaluable for identifying viral pathogenesis determinants. In addition such an analysis would facilitate the development of roseolovirus diagnostics. Current PCR-based diagnostics for the assessment of viral load in patient samples vary greatly in sensitivity between laboratories [49]. This may reflect differences in the primers used for detection, methods of nucleic acid isolation and/or the choice of standardization protocols. However strain variability across different geographical regions could also account for these discrepancies. A thorough genomics analysis of strain variation for each roseolovirus would provide the starting point for the development of diagnostics targeting invariant regions of roseolovirus genomes. These data would also allow for the generation of suitable reference strains that are needed to standardize diagnostic assays.

The ability to efficiently map viral transcription in a tissue or viral lifecycle specific manner is crucial to understanding how these viruses grow and cause disease. Due to the reproducibility, low cost, high sensitivity and high specificity of tiled arrays [50], development of virus-specific arrays for HHV-6A, HHV-6B and HHV-7 should be prioritized. The production and use of tiled arrays is a standard commodity at most institutions and offers a unified platform for transcript characterization and quantification. In addition RNA-Seq and other next generation sequencing approaches can identify novel coding regions [51], small RNAs [52,53] and/or splice junctions [54] in herpesvirus genomes that may expand the roseolovirus proteome. A combinatorial transcriptomic approach is a priority for defining the coding capacity and regulatory regions of roseolovirus genomes.

As with functional genomics, the ability to perform directed proteomics experiments to define the function of HHV-6B and HHV-7 proteins is severely hampered by the lack of infectious BAC clones. However such approaches should be easily applicable to the study of HHV-6A, as the necessary genetic system exists and the required reagents are commercially available. In addition, mass spectrometry core facilities capable of producing and analyzing the data from proteomics experiments are common at most research institutions. As with transcriptomics, a combination of proteomics approaches is needed to define novel viral coding determinants and their functions in order to identify new targets for antiviral drugs.

Although metabolomics is less common than other -omics approaches in the study of herpesviruses, metabolomics has already identified virus-induced metabolic changes that can be targeted with drugs. Metabolic perturbations underlie several disease states, and the development of drugs that regulate metabolism is an active area of clinical research. In some cases, metabolomics studies have suggested that currently approved drugs have been found to have novel antiviral activity [47], potentially speeding translation of these findings into the clinic. Metabolomics approaches should be relatively straightforward and thus should be prioritized.

The rapid development of -omics approaches over the last ten years has greatly changed our view of herpesvirus biology. As described above, many standard -omics approaches can be easily adapted for roseolovirus research, although for studies of HHV-6B and HHV-7 some hurdles still remain. The lack of -omics approaches to date in roseolovirus research as a whole presents an opportunity for researchers to coordinate an integrated and highly targeted -omics analysis of these clinically important human pathogens.

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References

1. Li H: **Systems genetics in ''-omics'' era: current and future development**. *Theory Biosci = Theorie in den Biowissenschaften* 2013, **132**:1-16.
2. Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M, Halligan G, Biberfeld P, Wong-Staal F, Kramarsky B *et al.*: **Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders**. *Science* 1986, **234**:596-601.
3. Dominguez G, Dambaugh TR, Stamey FR, Dewhurst S, Inoue N, Pellett PE: **Human herpesvirus 6B genome sequence: coding content and comparison with human herpesvirus 6A**. *J Virol* 1999, **73**:8040-8052.
4. Gompels UA, Nicholas J, Lawrence G, Jones M, Thomson BJ, Martin ME, Efstathiou S, Craxton M, Macaulay HA: **The DNA sequence of human herpesvirus-6: structure, coding content, and genome evolution**. *Virology* 1995, **209**:29-51.
5. Megaw AG, Rapaport D, Avidor B, Frenkel N, Davison AJ: **The DNA sequence of the RK strain of human herpesvirus 7**. *Virology* 1998, **244**:119-132.
6. Gravel A, Ablashi D, Flamand L: **Complete genome sequence of early passaged human herpesvirus 6A (GS strain) isolated from North America**. *Genome Announc* 2013, **1**.
7. Isegawa Y, Mukai T, Nakano K, Kagawa M, Chen J, Mori Y, Sunagawa T, Kawanishi K, Sashihara J, Hata A *et al.*: **Comparison of the complete DNA sequences of human herpesvirus 6 variants A and B**. *J Virol* 1999, **73**:8053-8063.
8. **Human herpesvirus-6 strain groups: a nomenclature**. *Arch Virol* 1993, **129**:363-366.
9. Ablashi D, Agut H, Alvarez-Lafuente R, Clark DA, Dewhurst S, DiLuca D, Flamand L, Frenkel N, Gallo R, Gompels UA *et al.*: **Classification of HHV-6A and HHV-6B as distinct viruses**. *Arch Virol* 2014, **159**:863-870.
10. Adams MJ, Carstens EB: **Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses (2012)**. *Arch Virol* 2012, **157**:1411-1422.
11. Tang H, Kawabata A, Yoshida M, Oyaizu H, Maeki T, Yamanishi K, Mori Y: **Human herpesvirus 6 encoded glycoprotein Q1 gene is essential for virus growth**. *Virology* 2010, **407**:360-367.
12. Oyaizu H, Tang H, Ota M, Takenaka N, Ozono K, Yamanishi K, Mori Y: **Complementation of the function of glycoprotein H of human herpesvirus 6 variant A by glycoprotein H of variant B in the virus life cycle**. *J Virol* 2012, **86**:8492-8498.
13. Alba MM, Das R, Orengo CA, Kellam P: **Genomewide function conservation and phylogeny in the Herpesviridae**. *Genome Res* 2001, **11**:43-54.
14. Cha TA, Tom E, Kemble GW, Duke GM, Mocarski ES, Spaete RR: **Human cytomegalovirus clinical isolates carry at least 19 genes not found in laboratory strains**. *J Virol* 1996, **70**:78-83.
15. Goodrum F, Reeves M, Sinclair J, High K, Shenk T: **Human cytomegalovirus sequences expressed in latently infected individuals promote a latent infection in vitro**. *Blood* 2007, **110**:937-945.
16. Salsman J, Zimmerman N, Chen T, Domagala M, Frappier L: **Genome-wide screen of three herpesviruses for protein subcellular localization and alteration of PML nuclear bodies**. *PLoS Pathogens* 2008, **4**:e1000100.
17. Paladino P, Marcon E, Greenblatt J, Frappier L: **Identification of herpesvirus proteins that contribute to G1/S arrest**. *J Virol* 2014, **88**:4480-4492.
18. Song MJ, Hwang S, Wong WH, Wu TT, Lee S, Liao HI, Sun R: **Identification of viral genes essential for replication of murine gamma-herpesvirus 68 using signature-tagged mutagenesis**. *Proc Natl Acad Sci U S A* 2005, **102**:3805-3810.
19. Brune W, Menard C, Heesemann J, Koszinowski UH: **A ribonucleotide reductase homolog of cytomegalovirus and endothelial cell tropism**. *Science* 2001, **291**:303-305.
20. Dunn W, Chou C, Li H, Hai R, Patterson D, Stolc V, Zhu H, Liu F: **Functional profiling of a human cytomegalovirus genome**. *Proc Natl Acad Sci U S A* 2003, **100**:14223-14228.
21. Yu D, Silva MC, Shenk T: **Functional map of human cytomegalovirus AD169 defined by global mutational analysis**. *Proc Natl Acad Sci U S A* 2003, **100**:12396-12401.
22. Tsao EH, Kellam P, Sin CS, Rasaiyaah J, Griffiths PD, Clark DA: **Microarray-based determination of the lytic cascade of human herpesvirus 6B**. *J Gen Virol* 2009, **90**:2581-2591.
23. Yao K, Mandel M, Akyani N, Maynard K, Sengamalay N, Fotheringham J, Ghedin E, Kashanchi F, Jacobson S: **Differential HHV-6A gene expression in T cells and primary human astrocytes based on multi-virus array analysis**. *Glia* 2006, **53**:789-798.
24. van Dijk EL, Auger H, Jaszczyszyn Y, Thermes C: **Ten years of next-generation sequencing technology**. *Trends Genet: TIG* 2014.
25. Strong MJ, O'Grady T, Lin Z, Xu G, Baddoo M, Parsons C, Zhang K, Taylor CM, Flemington EK: **Epstein-Barr virus and human herpesvirus 6 detection in a non-Hodgkin's diffuse large B-cell lymphoma cohort by using RNA sequencing**. *J Virol* 2013, **87**:13059-13062.
26. Yozwiak NL, Skewes-Cox P, Stenglein MD, Balmaseda A, Harris E, DeRisi JL: **Virus identification in unknown tropical febrile illness cases using deep sequencing**. *PLoS Negl Trop Dis* 2012, **6**:e1485.
27. Tuddenham L, Jung JS, Chane-Woon-Ming B, Dolken L, Pfeffer S: **Small RNA deep sequencing identifies microRNAs and other small noncoding RNAs from human herpesvirus 6B**. *J Virol* 2012, **86**:1638-1649.
28. Fakhari FD, Dittmer DP: **Charting latency transcripts in Kaposi's sarcoma-associated herpesvirus by whole-genome real-time quantitative PCR**. *J Virol* 2002, **76**:6213-6223.
29. Cheng BY, Zhi J, Santana A, Khan S, Salinas E, Forrest JC, Zheng Y, Jaggi S, Leatherwood J, Krug LT: **Tiled microarray identification of novel viral transcript structures and distinct transcriptional profiles during two modes of productive murine gammaherpesvirus 68 infection**. *J Virol* 2012, **86**:4340-4357.
30. Rowles DL, Terhune SS, Cristea IM: **Discovery of host–viral protein complexes during infection**. *Methods Mol Biol* 2013, **1064**:43-70.
31. Cristea IM, Moorman NJ, Terhune SS, Cuevas CD, O'Keefe ES, Rout MP, Chait BT, Shenk T: **Human cytomegalovirus pUL83 stimulates activity of the viral immediate-early promoter through its interaction with the cellular IFI16 protein**. *J Virol* 2010, **84**:7803-7814.
32. Moorman NJ, Sharon-Friling R, Shenk T, Cristea IM: **A targeted spatial-temporal proteomics approach implicates multiple cellular trafficking pathways in human cytomegalovirus virion maturation**. *Mol Cell Proteom MCP* 2010, **9**:851-860.
33. Terhune SS, Moorman NJ, Cristea IM, Savaryn JP, Cuevas-Bennett C, Rout MP, Chait BT, Shenk T: **Human cytomegalovirus UL29/28 protein interacts with components of the NuRD complex which promote accumulation of immediate-early RNA**. *PLoS Pathog* 2010, **6**:e1000965.
34. Stahl JA, Chavan SS, Sifford JM, Macleod V, Voth DE, Edmondson RD, Forrest JC: **Phosphoproteomic analyses reveal signaling pathways that facilitate lytic gammaherpesvirus replication**. *PLoS Pathog* 2013, **9**:e1003583.

35. Becker GW: **Stable isotopic labeling of proteins for quantitative proteomic applications**. *Brief Funct Genom Proteomics* 2008, **7**:371-382.

36. Drissi R, Dubois ML, Boisvert FM: **Proteomics methods for subcellular proteome analysis**. *FEBS J* 2013, **280**:5626-5634.

37. Gudleski-O'Regan N, Greco TM, Cristea IM, Shenk T: **Increased expression of LDL receptor-related protein 1 during human cytomegalovirus infection reduces virion cholesterol and infectivity**. *Cell Host Microbe* 2012, **12**:86-96.

38. Stern-Ginossar N, Weisburd B, Michalski A, Le VT, Hein MY, Huang SX, Ma M, Shen B, Qian SB, Hengel H *et al.*: **Decoding human cytomegalovirus**. *Science* 2012, **338**:1088-1093.

39. Aldridge BB, Rhee KY: **Microbial metabolomics: innovation, application, insight**. *Curr Opin Microbiol* 2014, **19C**:90-96.

40. Delgado T, Sanchez EL, Camarda R, Lagunoff M: **Global metabolic profiling of infection by an oncogenic virus: KSHV induces and requires lipogenesis for survival of latent infection**. *PLoS Pathog* 2012, **8**:e1002866.

41. Munger J, Bajad SU, Coller HA, Shenk T, Rabinowitz JD: **Dynamics of the cellular metabolome during human cytomegalovirus infection**. *PLoS Pathog* 2006, **2**:e132.

42. Munger J, Bennett BD, Parikh A, Feng XJ, McArdle J, Rabitz HA, Shenk T, Rabinowitz JD: **Systems-level metabolic flux profiling identifies fatty acid synthesis as a target for antiviral therapy**. *Nat Biotechnol* 2008, **26**:1179-1186.

43. Abrantes JL, Alves CM, Costa J, Almeida FC, Sola-Penna M, Fontes CF, Souza TM: **Herpes simplex type 1 activates glycolysis through engagement of the enzyme 6-phosphofructo-1-kinase (PFK-1)**. *Biochim Biophys Acta* 2012, **1822**:1198-1206.

44. Spencer CM, Schafer XL, Moorman NJ, Munger J: **Human cytomegalovirus induces the activity and expression of acetyl-coenzyme A carboxylase, a fatty acid biosynthetic enzyme whose inhibition attenuates viral replication**. *J Virol* 2011, **85**:5814-5824.

45. Bhatt AP, Jacobs SR, Freemerman AJ, Makowski L, Rathmell JC, Dittmer DP, Damania B: **Dysregulation of fatty acid synthesis and glycolysis in non-Hodgkin lymphoma**. *Proc Natl Acad Sci U S A* 2012, **109**:11818-11823.

46. Vastag L, Koyuncu E, Grady SL, Shenk TE, Rabinowitz JD: **Divergent effects of human cytomegalovirus and herpes simplex virus-1 on cellular metabolism**. *PLoS Pathog* 2011, **7**:e1002124.

47. Rabinowitz JD, Purdy JG, Vastag L, Shenk T, Koyuncu E: **Metabolomics in drug target discovery**. *Cold Spring Harbor Symp Quant Biol* 2011, **76**:235-246.

48. Flamand L, Komaroff AL, Arbuckle JH, Medveczky PG, Ablashi DV: **Review, part 1: human herpesvirus-6-basic biology, diagnostic testing, and antiviral efficacy**. *J Med Virol* 2010, **82**:1560-1568.

49. Cook L, Atienza EE, Bagabag A, Obrigewitch RM, Jerome KR: **Comparison of methods for extraction of viral DNA from cellular specimens**. *Diagn Microbiol Infect Dis* 2009, **64**:37-42.

50. Hovik H, Chen T: **Dynamic probe selection for studying microbial transcriptome with high-density genomic tiling microarrays**. *BMC Bioinform* 2010, **11**:82.

51. Canny SP, Reese TA, Johnson LS, Zhang X, Kambal A, Duan E, Liu CY, Virgin HW: **Pervasive transcription of a herpesvirus genome generates functionally important RNAs**. *mBio* 2014, **5** e01033-01013.

52. Chugh PE, Sin SH, Ozgur S, Henry DH, Menezes P, Griffith J, Eron JJ, Damania B, Dittmer DP: **Systemically circulating viral and tumor-derived microRNAs in KSHV-associated malignancies**. *PLoS Pathog* 2013, **9**:e1003484.

53. Marquitz AR, Mathur A, Chugh PE, Dittmer DP, Raab-Traub N: **Expression profile of microRNAs in Epstein-Barr virus-infected AGS gastric carcinoma cells**. *J Virol* 2014, **88**:1389-1393.

54. Gatherer D, Seirafian S, Cunningham C, Holton M, Dargan DJ, Baluchova K, Hector RD, Galbraith J, Herzyk P, Wilkinson GW, Davison AJ: **High-resolution human cytomegalovirus transcriptome**. *Proc Natl Acad Sci U S A* 2011, **108**:19755-19760.