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REVIEW

Summary of the 11th International Conference on Human Herpesviruses-6A, -6B, and -7

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Abstract

The 11th International Conference on Human Herpesviruses (HHV)-6A, -6B, and -7 was held in Quebec City, from 23 to 26 June 2019. It attracted 144 basic, translational, and clinical scientists from 20 countries. Important new information was presented regarding: the mechanisms of chromosomal integration for HHV-6A and -6B; the biology of inherited chromosomally integrated HHV-6A and -6B; animal models for, and animal viruses with similarities to, HHV-6A, -6B, and -7; established and possible disease associations, including provocative new information suggesting these viruses may be one trigger of Alzheimer's disease, and new treatment strategies. In this review, we summarize presentations that were of particular interest. The full text of the Abstracts cited in the manuscript is available in the online Supporting Information Materials.

KEYWORDS

HHV-6A, HHV-6B, HHV-7, human herpesvirus-6, human herpesvirus-6A, human herpesvirus-6B, human herpesvirus-7, research conference

1 | INTRODUCTION

The 11th International Conference on Human Herpesviruses (HHV)-6A, -6B, and -7 was organized by two of us (LF and DZ) and was held in Québec City, QC, Canada, from 23 to 26 June 2019 with the support of the HHV-6 Foundation, Université Laval, CHU de Québec Research Center, and several corporate sponsors (see Acknowledgments).

The Conference attracted 144 basic, translational, and clinical scientists from 20 countries. There were a total of 89 oral and poster presentations of original research, in addition to several keynote and state of the art talks. In this manuscript we summarize the Abstract (s) by referencing their Abstract number; the full texts of the Abstracts are provided in the Supporting Information Materials.

2 | CHROMOSOMAL INTEGRATION OF HHV-6A AND -6B

HHV-6A and -6B can integrate their genomes into the telomeres of host chromosomes near the subtelomeric region. In contrast to other human herpesviruses, no viral episomes have been detected during latency. The integration process is facilitated by telomeric repeats present at the ends of the viral DNA. The viruses also can undergo excision from their integrated state (be reactivated), and then undergo lytic replication. The mechanisms by which integration (latency) and excision (reactivation) occur remain to be fully elucidated. It also is unclear whether the integrated virus can produce pathogenic proteins even when it does not produce new virions, as can occur with endogenous retroviruses.

The immediate-early protein 1 of HHV-6A and -6B (IE1A and B) is one of the first proteins expressed during infection, and is invariably associated with promyelocytic leukemia protein (PML). PML, in turn, is (a) involved in various cellular regulatory mechanisms such as DNA damage responses; (b) oligomerizes and forms nuclear bodies (PML-NBs) to recruit interacting partners; and (c) appears to be a key player in bringing SUMOylated IE1A and B to telomeres, the site of integration. **[Abstract 4-4]**

Histone modification occurs in regions affecting gene expression during the chromosomal integration of HHV-6A. Further investigation

is needed to determine the specific ways in which the histone modifications affect both the process of integration, transcriptional activation of viral genes, and the signaling of host genes. **[Abstract 4-9]**

Using CRISPR/Cas9, investigators achieved precise excision of the integrated HHV-6 genome from host telomeres in over 80% of cells, as determined by fluorescent in situ hybridization (FISH), quantitative polymerase chain reaction (qPCR), and fluorescence-activated cell sorting. This could become a tool for studying the mechanisms and biological effects of integration and excision. **[Abstract 4-10]**

3 | INHERITED CHROMOSOMALLY INTEGRATED HHV-6A AND -6B (iciHHV-6A AND -B)

3.1 | Background

Over many millennia, in independent events, both HHV-6A and -6B have integrated into the chromosomes of germline cells. As a consequence, approximately 1% of humans have inherited the entire viral genome from a parental germ cell, and the viral genome is present in every cell. This phenomenon is termed inherited chromosomally integrated HHV-6A and -6B (iciHHV-6A and -6B). The Conference produced preliminary information addressing several of the most interesting unanswered questions about iciHHV-6A and -6B.

3.2 | History of human integration events

In a population of 8498 Japanese people, 35 iciHHV-6A or -6B individuals were identified. Two independent events had led to the integration of the HHV-6A or -6B genome on chromosome 22q, and accounted for approximately 70% of all cases of iciHHV-6A or -6B in this sample. FISH revealed that each of the two integration events was tightly linked to a rare haplotype, strongly suggesting that two "founder effect" events occurred approximately 2300 years ago—during a period when a large infusion of continental Asians settled in Japan. **[Abstract 4-6]**

Previous research revealed that the prevalence of iciHHV-6A or -6B was approximately 2.5% in Scotland and approximately 1% in England. Interestingly, the prevalence was higher than 1% among people of Scottish ancestry living in England, and lower than 2.5% among people of English ancestry living in Scotland. A new report from Scotland, involving 24 000 people, reported a prevalence of 2.7%. Bioinformatics analysis suggested that there were 10 distinct ancestral iciHHV-6B lineages, all located on chromosomes 9q, 11p, 17p, or 19q. **[Abstract 4-16]**

Investigators sequenced 238 individuals with iciHHV-6A or -6B, from multiple geographic regions. They identified at least a dozen independent integration events, the first of which occurred before *Homo sapiens* first migrated out of Africa. Although the investigators confirmed that the sequences of iciHHV-6A and -6B are quite different from the sequences of community strains, some iciHHV-6B sequences closely resemble those of circulating strains of HHV-6B, suggesting that contemporary germline integration is also occurring. **[Abstract 4-8]**

3.3 | Transcriptional activity of the integrated viral sequences

Many human endogenous retroviral sequences are transcriptionally inactive. Is the same true for iciHHV-6? Several small molecules were used to stimulate the reactivation of iciHHV-6B in peripheral mononuclear cells. Phorbol 12-myristate 13-acetate led to substantial increases in HHV-6B gene expression, with a dose-response effect. As have others, this study confirms that iciHHV-6B can become transcriptionally active, with appropriate stimulation. **[Abstract 4-7]**

Expression of HHV-6A and -6B genes in people with iciHHV-6A or -6B was found in many tissues: brain, testis, breast, adrenal gland, lungs, salivary gland, esophagus, skeletal muscle, colon, tibial nerve and artery, adipose tissue, heart, skin, and thyroid. Interestingly, iciHHV-6A was predominantly expressed in brain tissue, whereas no such predominance in any organ was seen for iciHHV-6B. The highest expression levels for both iciHHV-6A and -6B were found in the U90 and U100 genes. There was no HHV-6 RNA expression found in any of the non-iciHHV-6 controls. **[Abstract 4-5]**

3.4 | Differences in the immune response to iciHHV-6A and -6B vs infection with community strains

Investigators screened 15 498 subjects and identified 46 (54%) as iciHHV-6A and 43 (46%) as iciHHV-6B. These subjects were compared with control subjects matched for age and sex. The cases and controls were no different with regard to (a) levels of 14 plasma cytokines; (b) antibodies to Epstein-Barr virus (EBV), and human influenza virus; or (c) T-cell responses against EBV, cytomegalovirus (CMV), or influenza antigens. However, iciHHV-6A and -6B individuals had higher anti-CMV antibody levels than controls. Furthermore, iciHHV-6A subjects had more robust antibody responses to the following antigens: U11A, U11B, and IE1A. Similarly, iciHHV-6B subjects had a more robust antibody response against IE1B and IE1A antigens. **[Abstract 4-2]**

3.5 | Excision of the integrated viral genome

The consequences for telomeres when iciHHV-6A and -6B genomes are partially or completely released from their integrated state are different in different cell lines—lymphoblastoid, circulating mononuclear cells, and sperm. The consequences for telomeres also depend on the position of the integrated virus in the telomere. In particular, truncations at DR_L-T2 (short array repeats) lead to very short telomeres capable of initiating a DNA damage response. Occasionally, investigators found telomeres that were unusually long; they speculate that the excision event

may have occurred in a hematopoietic stem cell, in which telomerase is active. **[Abstract 4-3]**

3.6 | Sequences of iciHHV-6A and -6B vs community strains

Investigators sequenced 109 community strains of HHV-6A and -6B from hospital patients, as well as the viral sequence in 104 subjects with iciHHV-6A or -6B. As has been noted previously, whereas community strains were overwhelmingly HHV-6B (94%) only 58% of iciHHV-6 cases were iciHHV-6B. While both iciHHV-6A and -6B sequences shared some sequence similarities with community strains, the iciHHV-6B sequences displayed much less variability than community strains. **[Abstract 4-12]**

Unfortunately, this year's conference offered no further information on the adverse (or beneficial) health consequences in individuals born with iciHHV-6A or -6B.

4 | BIOLOGY OF HHV-6A AND -6B INFECTIONS

4.1 | Immune response to natural infection, and implications for vaccines

HHV-6A enters cells after its tetrameric envelope glycoprotein complex (gH/gL/gQ1/gQ2) binds to human CD46, which is expressed on almost all cell types. Investigators expanded upon their previous work demonstrating that the equivalent complex for HHV-6B binds to human CD134, which is specifically expressed on activated T cells. The team expressed and purified both ligand and receptor, and then studied the ultrastructure of each using negative stain electron microscopy. Specific neutralizing monoclonal antibodies bind to specific parts of the tetrameric complex, thereby inhibiting binding of the virus to the receptor. **[Abstract 6-1]**

In mice, the glycoprotein complex, or portions of the complex, induced both the production of specific antibodies and specific cellular immunity. Importantly, sera of immunized mice carried neutralizing antibodies suggesting that gQ1 and gQ2 could represent potential components of an eventual HHV-6B vaccine. **[Abstract 7-1]**

"Humanized" chimeric monoclonal antibodies were created that contained the heavy and light chain variable regions of KH-1 (anti-gQ1) and OHV-3 (anti-gH). These humanized antibodies retained specificity for their antigenic targets and were as potent in neutralizing infection with HHV-6B as were the mouse antibodies from which they were derived. This could prove to be a step in the road toward a prophylactic therapy against HHV-6B. **[Abstract 8-3]**

Residues 130 to 185 of the AgQ2 component of the HHV-6A tetrameric complex were found to be critical in binding to and stabilizing CD46. This finding could prove to be a step in the road toward a vaccine against HHV-6A. **[Abstract 8-4]**

T-cell populations responding to HHV-6B in healthy donors were mainly CD3+ CD4+ from the effector memory ($CD45RA^-/CCR7^-$) subset. Responding cells produced interferon γ (IFN-γ), tumor necrosis factor α, and low levels of interleukin 2 (IL-2), and killed target cells in vitro. The epitopes targeted by the T-cell response included virion structural components as well as other viral proteins. **[Abstract 7-2]**

A protein essential for signaling through the T-cell receptor (tyrosine phosphatase CD45) is downregulated by infection with HHV-6A and -7. Through the deletion of specific viral open reading frames (ORFs), the investigators showed that ORFs U21 to U24 was necessary for this downregulation of CD45. **[Abstract 7-4]**

4.2 | Miscellaneous observations about HHV-6 biology

HHV-6A encodes a small noncoding RNA (sncRNA), sncRNA-U14, that targets another sncRNA, miR-30. Further work will be required to better understand the interaction of sncRNA-U14 and miR-30 and its effect on protein translation. **[Abstract 8-2]**

Infection of T cells with both HHV-6A and -6B increases both mitochondrial replication and mitochondrial DNA transcription. It also alters the expression ratio of nuclear/mitochondrial OXPHOS genes, resulting in a decreased mitochondrial membrane potential, an increase in the production of reactive oxygen species and an increase in glycolysis. **[Abstract 3-9]**

After infecting Molt-3 T cells with HHV-6B (Z29 strain), serial measurements were made of the transcriptome over 72 hours. As expected, expression of new HHV-6B transcripts was identified at all time points. Interestingly, many transcripts represented spliced variants of previously reported transcripts. Analysis is underway of the consequences for protein translation. **[Abstract 6-3]**

Previous reports have linked HHV-6A infection of the endometrium to primary infertility of unknown cause. Women with such infertility, and having an endometrial infection with HHV-6A, were compared with infertile women negative for HHV-6A infection. The endometrium of the former group was characterized by activated natural killer cells and diminished numbers of regulatory T cells. These immunological changes were shown to reduce the receptivity of endometrial epithelial cells to implantation. Studies of a comparison group of fertile women were not reported. **[Abstract 7-3]**

5 | ANIMAL MODELS

Progress was reported with animal models of HHV-6 biology. Transgenic mice with the human CD46 receptor that were infected with HHV-6A developed persistent brain infection (viral DNA remained detectable for at least 15 months), and the brain became infiltrated with immune cells. The animals developed impairments in behavioral tests regarded as proxies for a depressive phenotype and a fear response. At autopsy, the virus appeared to be integrated into host telomeres and was particularly likely to target Purkinje cells in the cerebellum and raphe nuclei, important parts of the serotonergic pathway. Interestingly, a recent study

reported finding infection of Purkinje cells by HHV-6A in people with bipolar disorder. **[Abstract 2-1]**

Murine roseolovirus—previously named mouse thymic virus (MTV)—is a mouse homolog of HHV-6A, -6B, and -7. Investigators performed minor histocompatibility antigen-mismatched bone marrow transplants in latently infected mice and demonstrated reactivation leading to lytic infection in the skin, liver, and lungs (idiopathic pneumonia syndrome-like pathological picture). The degree of reactivation also correlated with the severity of acute graft-vs-host disease (aGVHD) in the mice. **[Abstract 2-2]**

Previous research has linked neonatal infection of mice with MTV to the production of a wide variety of autoantibodies and to the development of autoimmune gastritis. Investigators reported that neonatal infection also causes autoimmune gastritis in multiple mouse strains presenting in adult life. It is preceded by a transient reduction in thymic and peripheral regulatory T cells and is characterized by a mucosa infiltrated by T cells, neutrophils, and eosinophils infiltration as well as the absence of the detectable virus. It is inhibited by treatment with ganciclovir early in life. **[Abstract 2-3]**

Porcine cytomegalovirus (PCMV) also has considerable homology with HHV-6A, -6B, and -7, making the pig another potential animal model for the study of roseoloviruses. When pig organs are transplanted into primates during xenotransplantation studies, PCMV disseminates widely: viral DNA and proteins are detectable in many primate organs. Graft survival is reduced by PCMV infection. **[Abstract 2-4]**

6 | HHV-6A AND -6B AND MALIGNANCY

The possible role of HHV-6A and -6B in malignancy remains uncertain. In contrast to EBV and Kaposi's sarcoma-associated herpesvirus (HHV-8), which often are found within tumor cells and which produce oncoproteins, reports of the presence of HHV-6A and -6B DNA or proteins within tumor cells have been inconsistent. Several presentations at the conference suggested that the possible role of HHV-6A and -6B in oncogenesis more likely involves the tumor microenvironment rather than cellular transformation.

Reed-Sternberg cells and associated lymphoid cells from primary Hodgkin lymphoma were found to have very high expression of the U51 and U24A transcripts of HHV-6B within the associated lymphoid cells, but not within tumor cells. The expression of these transcripts is likely to affect cytokine production, although the investigators did not report the results of cytokine production. Subsequent immunohistochemical studies of different types of lymphoid malignancies linked HHV-6B to T-cell lymphomas, but not to diffuse large B cell and follicular lymphomas. **[Abstract 5-2]**

An important latency gene for HHV-6A and -6B, U94, has three effects of potential relevance for cancer biology. First, it strongly inhibits angiogenesis. Second, it induces a partial mesenchymal-to-epithelial transition: it thereby impairs cell migration, invasion, and proliferation. Third, it also inhibits genes, such as BRCA1, involved in DNA repair. When the U94 protein is added to the chemotherapy drugs used to treat triple-negative breast cancer, in vitro, it has a chemosensitizing effect. **[Abstract 8-5]**

In addition, the upregulation and release of human leukocyte antigen G (HLA-G) are induced by HHV-6A and -6B infection of endothelial cells, directly inhibiting angiogenesis. **[Abstract 8-6]**

7 | HHV-6 AND DRUG REACTION WITH EOSINOPHILIA AND SYSTEMIC SYMPTOMS/DRUG-INDUCED HYPERSENSITIVITY SYNDROME

Reactivation of HHV-6B has been linked to drug reaction with eosinophilia and systemic symptoms (DRESS)/drug-induced hypersensitivity syndrome (DIHS). This illness is characterized by fever, cutaneous eruptions, hematological abnormalities, dysfunction of various organs, and reactivation of HHV-6B. High levels of HHV-6B DNA were found in whole blood or peripheral blood mononuclear cells in approximately 20% of patients with DRESS/DIHS: these patients had more severe lesions of their skin and mucous membranes, higher levels of HHV-6B DNA during the acute phase of the illness, higher rates of concomitant human cytomegalovirus (HCMV) reactivation, higher levels of IL-4 during the acute phase and of soluble IL-2 receptor in the late phase, and a higher rate of long-term complications (such as nephritis, arthritis, and thyroiditis). It remains unclear whether HHV-6B has a causal role in DRESS/DIHS, or whether the findings are an epiphenomenon of more severe illness. **[Abstract 11-1]**

One possible mechanism by which HHV-6A and -6B (or any virus) might work in conjunction with HLA proteins to encourage hypersensitivity reactions involves heterologous immunity: T-cell receptors of virus-primed T cells bind to HLA antigens that are associated with a drug metabolite, the novel tertiary structures eliciting an immune response. For example, the presence of HHV-6 DNA in whole blood and the HLA-B*13:01 together raise the risk of hypersensitivity to trichlorethylene nearly 100-fold. **[Abstract 11-2]**

8 | HHV-6 AND TRANSPLANTATION

Reactivation of HHV-6B is a complication of hematopoietic cell transplantation (HCT), particularly in umbilical cord blood transplantation (UCBT). Reactivation of HHV-6B is correlated with various adverse outcomes including limbic encephalitis (the limbic system includes the hypothalamus, hippocampus, and amygdala), idiopathic pneumonia, acute aGVHD and lower rates of 1-year survival. Interpretation of the literature on HHV-6B and HCT is complicated by the inevitable variability inherent in studies of HCT: different clinical conditions for which HCT is being performed, different ages of the graft recipients, different conditioning regimens, different types of cells transplanted, and different assays (of varying sensitivity) for assessing HHV-6 viremia. **[Abstract 9-1]**

The initial phase of T-cell reconstitution depends on the expansion of T cells from the graft. However, longer-term reconstitution requires thymopoiesis—the processing in the thymus of donor-derived lymphoid-myeloid progenitors leading to the production of naïve T lymphocytes. The vigor of thymopoiesis may be assessed by monitoring the levels of T-cell receptor excision circles (TRECs). In 28 patients who received UCBT, HHV-6B reactivation occurred in 86%; 25% of these patients developed HHV-6B-related encephalitis. Encephalitis was more likely in those with (a) the highest viral load; (b) lower TREC levels; and (c) lower CD4+ T-cell counts. **[Abstracts 9-2 and 9-3]**

HCT conditioning regimens that achieve T-cell depletion are associated with persistent HHV-6B viremia in a substantial fraction (27%) of patients, and approximately 8% of these develop the end-organ disease. Persistent viremia also is associated with lower overall 1-year survival. **[Abstract 9-5]**

One team reported initial reactivation of HHV-6B in 68% of allogeneic HCT. Reactivation was more likely in those patients with relatively high percentages of CD4+ T cells bearing CD134 (a receptor for HHV-6B) before transplantation, as well as in HLA-mismatched donors. **[Abstract 9-6]**

In contrast, other investigators found a low incidence (7%) of HHV-6B reactivation in HLA-haploidentical HCT (with a methylprednisolone/anti-thymocyte globulin conditioning regimen) as well as low levels of proinflammatory cytokines and an absence of HHV-6B-related encephalitis. The investigators suspect that since HHV-6 reactivation typically follows IL-6 reactivation, the low reactivation rate could be due to corticosteroid-induced suppression of IL-6. **[Abstract 9-7]**

A large observational study of HHV-6B-related lung disease found HHV-6B RNA in the bronchoalveolar lavage (BAL) fluid of 27% of HCT recipients with suspected lower respiratory tract disease. HHV-6B-positive subjects (regardless of copathogens in the BAL fluid) had significantly increased the risk of mortality and death from respiratory failure, compared with HHV-6B-negative subjects. Subjects who had received antivirals with efficacy against HHV-6B in the 3 days before BAL had a lower risk of mortality, compared with those not receiving antivirals. **[Abstract 9-8]**

Among 738 allogeneic HCT patients with HHV-6B reactivation within the first 100 days posttransplant, the risks of idiopathic pneumonia was fivefold higher than in patients without reactivation. In a murine HCT model, murine roseolovirus produced a similar pathologic phenotype in the lung. **[Abstract 9-9]** Similar results in mice were reported in Abstract 2-2.

Twenty pediatric patients with HCT were followed with serial magnetic resonance imaging (MRI) scans and assessments of HHV-6B reactivation. Eight (40%) had HHV-6B reactivation, of whom two had clinical evidence of encephalopathy (no encephalopathy was seen in the 12 patients without HHV-6B reactivation). In subjects with HHV-6B reactivation, initial inflammatory changes in the hippocampus were followed by reduction, over time, in the volume of the right hippocampus. **[Abstract 9-10]**

Among 33 patients undergoing HLA-matched, non-T-cell depleted, myeloablative allogeneic HCT, those with a higher number of donor CD4+ T cells, or a higher number of HHV-6B-specific donor memory donor T cells, had a reduced risk of HHV-6B reactivation. This result suggests that a strategy of enriching grafts with HHV-6B-specific T cells might confer clinical benefit in high-risk patients, such as those receiving UCBT or T-cell depleted grafts. **[Abstract 7-6]**

9 | HHV-6 AND LIVER DISEASE

Investigators conducted a retrospective review of eight cases of acute liver failure of unexplained cause requiring transplantation, in children aged 13 months to 14 years. Pathological examination revealed HHV-6 antigen (assay did not distinguish HHV-6A from -6B) in the glandular epithelium bile ducts and degenerating hepatocytes in all cases. The investigators speculate that the early identification of possible HHV-6 infection in such children, followed by antiviral therapy, might sometimes preserve the native liver and avert the need for transplantation. **[Abstract 9-11]**

10 | HHV-6A AND -6B AND CENTRAL NERVOUS SYSTEM DISEASE

10.1 | Multiple sclerosis

The hypothesis that multiple sclerosis (MS) might be triggered by neurotropic viruses was first posed shortly after the disease was described by Charcot in the 19th century. Yet many candidate viruses have failed the test of time. In the past 20 years, evidence has grown that EBV, HHV-6A, and HHV-6B might all be triggers of MS.

When HHV-6A engages specific extracellular domains of its receptor (CD46), it stimulates the multiple sclerosis-associated retroviruses, a member of the human endogenous retrovirus-W family, to express its proinflammatory envelope protein. The same does not occur with HHV-6B or measles virus (vaccine strain). **[Abstract 3-2]**

Forty patients with relapsing-remitting MS (RRMS) were compared to 40 with systemic lupus erythematosus and 40 healthy controls. Patients with RRMS had higher levels of immunoglobulin G (IgG) against HHV-6 U24A peptide than controls, in both plasma and cerebrospinal fluid (CSF). When T cells from patients with MS were stimulated in vitro with U24A peptide, they produced significantly higher amounts of IFN-γ. **[Abstract 3-3]**

Several studies in recent years have argued that MS is not only a disease of increased demyelination but also of decreased remyelination. Remyelination is generated by oligodendrocytes, which develop from oligodendrocyte precursor cells (OPCs). One potential latency-associated transcript of HHV-6A, encoding U94A, impairs the migration of human OPCs to sites requiring remyelination. **[Abstract 3-4]**

A remarkably large study (8742 patients with MS and 7215 matched healthy controls) measured IgG levels against several immediate-early proteins of HHV-6A and -6B. Elevated IgG levels against IE1 from HHV-6A were positively associated with MS (OR = 1.55, $P = 9 \times 10^{-22}$). The investigators then tested a separate cohort of 478 patients with MS from

whom serum was available before they developed MS (matched to 476 healthy controls). In this pre-MS cohort, IE1A-IgG also was strongly associated with the risk of subsequent MS (OR = 2.22, $P = 2 \times 10^{-5}$). No positive associations were noted with antibodies to HHV-6B early antigens. **[Abstract 3-5]**

When patients with MS were compared to people with other neurological diseases, of matched age and gender, no differences were found in the expression level of specific microRNAs synthesized by HHV-6A and -6B, in either serum or CSF. **[Abstract 3-6]**

Both HHV-6 and EBV have been linked to MS. HHV-6A can infect and replicate in EBV-positive B cell lines. When some T-cell lines are first infected with HHV-6A, CD21 (the receptor for EBV) is upregulated, leading to dual infection of the cells if they then are exposed to EBV. The investigators followed different dually infected cell lines—T cell, B cell, and glial cell lines—for up to 10 years. Curiously, since the primary immune cell target of EBV is B cells, dually infected T-cell lines lost the HHV-6A genome after several months, but the lines retained EBV and continued to express EBNA-1, EBNA-2, EBNA-3. In contrast, dually infected glial cell lines lost the EBV genome after a few weeks but continued to express HHV-6A proteins (although there was no evidence of viral replication). **[Abstract 6-2]**

10.2 | Alzheimer's disease

There is growing evidence that inflammation plays an important role in Alzheimer's disease (AD) pathology, with chronic brain infection by neurotropic agents being one plausible cause of chronic neuroinflammation. Reports in 2018 linking HHV-6A and -7 to AD generated several conference presentations.

One team summarized its study of brain tissues from patients with AD, other neurological diseases and healthy controls, using unbiased methods of DNA and RNA sequencing. In the brains of patients with AD they more often found evidence of increased DNA and RNA from HHV-6A, -7, Herpes simplex virus (HSV), and several other infectious agents. DNA and RNA loads for HHV-6A correlated strongly with clinical dementia scores, number of neurons, and density of amyloid-β (Aβ) plaques (a pathologic hallmark of AD). The findings were similar in three independent cohorts involving more than 1000 subjects. **[Abstracts 1-0 and 1-1]**

A second-team reported that, in mice and in human cell cultures, infection with HHV-6A, HHV-6B, or HSV-1 dramatically accelerates the deposition of Aβ, which then "entraps" the reactivated pathogens and prevents them from infecting other brain cells—ie, Aβ is a natural antimicrobial. However, over many years, the deposition of Aβ is associated with neurodestruction and AD. This evolutionarily disadvantageous aspect of Aβ biology might have been inapparent until the 20th century, given that the average lifespan even in developed nations was 60 years or less, and that noninherited forms of AD typically begin at a later age. **[Abstract 1-2]**

Other investigators presented evidence that two viruses, respiratory syncytial virus (RSV) and HSV-1 (HHV-6A and -7 were not studied) accumulate coronas made of human proteins present in biological fluids. These coronas seem to catalyze Aβ deposition. **[Abstract 1-3]**

Another team conducted a histological study of postmortem olfactory nerves in patients with mild cognitive impairment, moderate AD, and severe AD. Deposition of Aβ and particularly deposition of τ protein (another pathological hallmark of AD) was visible in the olfactory nerves: the greater the deposition, the greater the intellectual impairment. The same was true of HHV-6A late antigens (gp82), which colocalized with τ antigens. **[Abstract 1-4]**

Other investigators reported that HHV-6A caused a productive infection of microglial cells, which increased the expression of three AD risk factors: Aβ(1-40), τ protein, and APOE4 protein. **[Abstract 1-5]**

However, in contrast to the reports above, another team that used sensitive and specific digital droplet PCR techniques for detecting HHV-6A DNA and RNA found no clear differences in the brains of patients with AD vs healthy control subjects. **[Abstract 3-1]**

While the evidence suggesting that HHV-6A and -7 may be capable of triggering AD is exciting, important questions about the underlying biology remain to be answered, such as: in which cells is the viral DNA and RNA found; how many cells harbor viral genomes, and how does that relate to abundance of viral transcripts; which viral proteins do the cells express; how is viral activity linked to alterations of host gene transcription and do both happen in infected cells; do infected cells release cytokines or exosomes that modulate surrounding cells?

11 | HHV-6A AND -6B TREATMENT

No antiviral agent or immunotherapy has been specifically licensed to treat or prevent HHV-6A and -6B-associated diseases. While ganciclovir and foscarnet have antiviral activity against HHV-6B, as yet there is insufficient evidence of their value in pre-emptive or prophylactic treatment against HHV-6B-related encephalitis in patients with HCT. Oral brincidofovir has in vitro activity against both HHV-6B and HCMV and was evaluated in a randomized trial to test its efficacy in preventing complications from HCMV reactivation. Stored sera from the study allowed an assessment of its ability to prevent HHV-6B viremia, as well. Oral brincidofovir reduced the cumulative incidence of HHV-6B viremia at high risk, but not low risk, patients. The study was too small to assess its ability to prevent HHV-6B-related encephalitis. **[Abstract 10-2]**

Promising in vitro efficacy of several newer anti-herpesvirus drugs was reported—nucleoside analogs, nucleotide analogs, prodrugs (such as brincidofovir), drugs directed as the helicase/primase complex and various protein-protein interactions, and inhibitors of protein kinase and of DNA cleavage and packaging. Other new drugs target cellular proteins essential for viral replication (eg, inhibitors of cyclin-dependent kinases and the proteasome). Finally, several drugs approved for other purposes have been found to have activity against HHV-6A and -6B—eg, leflunomide, artesunate, sirolimus, and everolimus. **[Abstract 10-3]**

12 | FUTURE DIRECTIONS AND CHALLENGES

12.1 | Advances

Valuable new information was presented at the conference about the mechanisms of chromosomal integration, the immune response to infection, progress toward vaccine development, new animal models, new antiviral therapies, and the epidemiology and history of iciHHV-6A and -6B. Important information also was presented with regard to the possible pathogenic role of these viruses in post-HCT pneumonitis as well as in DIHS/DRESS, primary infertility, and MS. Provocative new evidence also suggested that HHV-6A and -7 infections of the brain may be one potential trigger of AD.

12.2 | Challenges

While considerable progress has been made in understanding the mechanisms of viral integration into the chromosome, much remains to be learned about the forces that affect latency vs reactivation. More needs to be learned about the possible adverse (or beneficial) health consequences of iciHHV-6A and -6B. Clinical trials of newer antivirals are in order, to determine their role in preventing and treating reactivated HHV-6B infection in patients with HCT, and the clinical consequences of doing so.

It remains challenging to firmly establish a causal relationship between these viruses and any particular disease, given that approximately 95% of humans develop a lifelong infection with HHV-6B in the first years of life. Nevertheless, growing evidence indicates these viruses may be a potential trigger for several diseases.

Overall, the steady progress that has been made since the 10th International Conference in 2017 suggests a bright future for research on these viruses.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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