Kurt Nikaitani

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Virology

Baker

Human Herpesvirus 6

Human herpesvirus 6 (HHV-6) is a DNA containing virus belonging to the *Roseolovirus* genus of the ß-herpesvirus subfamily. HHV-6 exists in two different variants referred to as A and B. HHV-6 has a worldwide distribution and presents itself as a typical herpesvirus often inducing latent infections in monocytes and macrophages. Infection with HHV-6 is extremely common, with nearly 100% in seroprevalence. The virus has a tropism for lymphocytes, often targeting CD4+ cells. It is likely that the virus is transmitted via oral secretions and in one study 90% of infected adults displayed virions in their saliva. It should be noted, however, that other studies have shown lower rates (Campadelli, Mirandola, & Menotti, 1999).

A natural HHV-6 infection can be divided into 3 stages. The first stage is the acute primary infection and this is most commonly seen in infants. HHV-6 is common in infants and about half of the children afflicted with the virus suffer the disease roseola infantum, exanthem subitum, or sixth disease. This is a mild childhood disease that produces symptoms such as rash and fever for 3-5 days. Adults rarely suffer primary infections since most people are infected as infants, but when it does occur symptoms are generally much more severe. The second occurs in health children as well as adults. During this stage the virus replicates in the salivary glands and is expelled in saliva while causing no noticeable

symptoms or pathology. The virus remains latent in, at least, lymphocytes and monocytes and replicates in low-levels in various tissues throughout the body. The third stage is less common, usually only seen in immunocompromised patients, and is known to cause reactivation of the virus from latency or reinfection (Campadelli, Mirandola, & Menotti, 1999).

Much attention was drawn to HHV-6 when light was shed on its ability to cause disease in immunosuppressed patients, particularly those suffering from AIDS. Primary or reoccurrence of infection in immunosuppressed people or suffers of AIDS can be life threatening. In fact, HHV-6 was first isolated in 1986 from "interleukin 2-stimulated peripheral blood mononuclear cells of patients with AIDS or lymphoproliferative disorders"(CDC 1999). In these patients, HHV-6 primary infection or reactivation can cause bone marrow suppression, encephalitis, pneumonitis, encephalopathy, hepatitis, rash, fever, and can even interfere with organ transplants resulting in rejection or death (Campadelli, Mirandola, & Menotti, 1999). Since more people are undergoing organ transplants, and thus must undergo therapeutic immunosuppression, the number of people at risk is increasing.

Furthermore, HHV-6 is one of the most neurotropic viruses in existence. Neruoinvasion has been seen in infants with primary infections, in focal encephalitis, patients suffering from AIDS, in those who have had bone marrow transplants, and even in immunologically competent children and adults. Both HHV-6A and HHV-6B have been discovered in the brain of patients who died of viral and non-viral causes. This demonstrates that both types of the virus can invade and inhabit the brain. Typically,

HHV-6B can result in CNS invasion, while HHV-6A has never been documented to do so (Campadelli, Mirandola, & Menotti, 1999).

There is also a possible link between an active HHV-6 infection and multiple sclerosis, a severe CNS disease that often afflicts young adults. It is characterized by demyelination of nerves that eventually results in complete paralysis and death. Multiple sclerosis appears to be an autoimmune reaction to myelin and its connection to HHV-6 is still inconclusive.

Recent Research in HHV-6 Replication

The mechanisms of HHV-6 in its involvement of development of diseases such as multiple sclerosis, epilepsy and others are yet to be completely understood, but a recent study has revealed that the HHV-6 encoded glycoprotein Q1 gene is necessary for virus growth (Tang et. al 2010). Glycoproteins and the complexes that they form on the surface of enveloped viruses are involved in the binding and entry of viruses into a host cell. The gQ proteins are exclusive to HHV-6 and HHV-7. The gQ1 protein of HHV-6 forms a complex with gH and gL, which subsequently binds to CD46, a cellular receptor for HHV-6. The researchers "speculated that gQ1 is essential for HHV-6 propagation, based on the function of gD in HSV-1, gp42 in EBV, and UL123-132 in HCMV," but lacked the data to prove this hypothesis (Tang et. al 2010). Due to advancements in biologic research tools, however, various human herpes viruses have been effectively cloned into F plasmids as BACs. This technique created the ability to stably maintain a viral genome as a BAC in *E. coli* as well as the mutagenesis of the viral genome via bacterial recombination machinery in the *E. coli* (Tang et. al 2010).

Tang et. al (2010) successfully cloned the HHV-6A U1102 genome as BAC plasmid and stably maintained the HHV-6A BAC in *E. coli.* Then the virus was reconstituted via transfection into a T-cell line and co-cultured with umbilical blood mononuclear cells (CBMCs). The viral protein expression was analyzed by Western blotting and the reconstituted virions were identified and confirmed through electron microscopy. The result was the generation of a deletion mutant of the gQ1 gene in the BAC genome. This mutation culminated in the failure to reconstitute the virus, thus suggesting that gQ1 was vital for HHV-6A propagation (Tang et. al 2010). Such research on the viral replication cycle of HHV-6 is essential in understanding the mechanisms of the virus. This can be used, in turn, to create more effective treatments.

HHV-6 and Multiple Sclerosis

Lately there has been an increase in interest in the possible role of HHV-6 in the development of multiple sclerosis (MS), but no virus has been definitively identified as a direct cause of MS. Certain HHVs, however, have been associated with the development of MS due to their ability to induce neurotropic behavior and institute latency. Human herpesvirus 6 is a likely candidate because it is neurotropic, primary infections often result in several neurological disorders, it is lymphotropic with immunomodulating properties, it possesses the ability to induce latency and periodic reactiviation, and it is ubiquitous (Voumcourakis, Kitos, Tsiodras, Pettrikos, & Stamboulis, 2010). Mechanisms of HHV-6 in the development of MS have been proposed including direct cytopathic action, molecular mimicry, and an increase in an existing immune response during virus reactivation, a phenomenon referred to as the bystander effect.

For example, Noseworthy (2003) discussed the possibility that molecular mimicry may explain the apparent link between HHV-6 and MS without directly connecting the virus in causing the disease. Due to the molecular similarity of the virus and myelin antigens it is possible that there is an immunological cross-reactivity between HHV-6 and myelin antigens. This would result in the demyelination of nerves that is characteristic of MS.

Another study aimed to identify the link between the amount of CD46 expression and the existence and viral load of HHV-6 in patients suffering from MS. In 1999, CD46 was identified as the primary receptor for HHV-6A and B (Alvarez-Lafuente, Garcia-Montojo, Dominguez-Mozo, Bartolome, & Arroyo, 2009). It was previously demonstrated that during the course of a HHV-6 infection CD46 was selectively and progressively down regulated from the target cell surface. The down-modulation of CD46, a complement regulation protein may cause complement activation and consequently would increase the susceptibility of the cell to complement lysis (Alvarez-Lafuente et. al, 2009).

There are two forms of CD46: membrane and soluble (sCD46), but there is no solid evidence that supports whether post-translational events or alternative splicing yields sCD46, whose levels have been demonstrated to increase in patients afflicted with autoimmune disorders such as MS (Alvarez-Lafuente et. al, 2009). It has been shown that a physical association amongst HHV-6 virion and sCD46 was found in the serum of MS patients with HHV-6 DNA, but was not present in the serum of controls, suggesting that the occurrence of HHV-6/CD46 complexes may be causative to the increased levels of CD46 seen in the patients with MS (Fogdell-Hahn, Soldan, & Shue, 2005). Alvarez-Lafuente et. al

(2009) purposed that there was a link between the levels of CD46 expression and the presence of HHV-6 virions in patients with MS.

To test this hypothesis blood and serum samples of 103 patients with MS were collected and the same number from healthy blood donors (controls) was also gathered. From these samples DNA and RNA was isolated and extracted from peripheral blood mononuclear cells and serum, and was then assessed via PCR. After identifying HHV-6 genomes and CD46 transcripts, the expression of rRNA18s was used to calculate the expression of CD46. Results indicated that nearly 80% of patients with MS had heightened levels of CD46 in comparison to controls. There was also a positive correlation between the HHV-6 viral load and the overall expression of CD46 in both the blood and serum. These findings suggest that the up-regulation of CD46 expression in patients with MS with HHV-6 infection could be due to an immunopathogenic factor involved in development of MS, but more research must be done to solidify these findings (Alvarez-Lafuente et. al, 2009).

In an attempt to establish whether there is a relationship between HHV-6 infection and MS, researchers reviewed the existing evidence and research that has already been gathered. A total of 61 studies were found and evaluated for quality using the Moor and Wolfson criteria and by the classification system employed by the Canadian Task Force on the Periodic Health Examination. Of the studies examined, 25 (41%) of 61 studies, 15 (60%) of which classified as high quality, reached what the researchers deemed as a statistically significant result (Voumvourakis, Kitsos, Tsiodras, Petrikkos, & Stamboulis, 2010). After the review was complete researchers suggested that the use of serology, which was the primary focus of many studies, to implicate HHV-6 in MS development

overstressed the positive result "without taking into consideration whether HHV-6 seropositivity is the result of a previous childhood infection or whether it is evidence of an ongoing infection that could be an active influential factor in MS pathogenesis" (Voumvourakis et. al, 2010).

Furthermore, a more reliable indication of "true infection and active presence of HHV-6" may be the use of PCR methods to identify HHV-6 specific DNA in the blood or CSF of MS patients (Voumvourakis, Kitsos, Tsiodras, Petrikkos, & Stamboulis, 2010). It should be noted, however, that even highly specific PCR can't accurately distinguish between HHV-6 reactivation from latency and a new infection. In regards to this issue, quantification of the exact viral load may be of assistance because acute infections are usually accompanied by higher viral loads. It would be beneficial if more studies examined the possible link between "new onset MS and the actual HHv-6 load present" because theoretically a "higher viral could lead to an increased immune response and a more pronounced neurological manifestation" (Voumvourakis et. al, 2010).

Voumvourakis et. al (2010) also proposed three conditions that could assist in the future design of studies attempting to shed light on the relationship between HHV-6 and MS. Firstly, controls should be similar as possible to patients with MS. Aside from age, sex, and race, it would be better to gather controls from the same population pools used for the MS patients. It has already been established that there is a worldwide prevalence of HHV-6 and it is necessary to gather more data on local prevalence and the populations that it affects. In addition, there is a possibility that HHV-6 infection may be associated with different rates of neurologic complications due to geography or genetic predisposition of

certain populations or individuals. Therefore, selection of controls from vast and diverse geographic populations may contribute to including controls that are genetically predisposed to MS, which may possibly harm the validity of the data (Voumvourakis et. al 2010).

The second condition was that the "diagnostic criteria of MS must be well defined and patients may have to be matched according to disease onset" (Voumvourakis, Kitsos, Tsiodras, Petrikkos, & Stamboulis, 2010). MS is a general term used to describe a wide range of clinical manifestations, which can be categorized as subtle, overt and serious progressive disease. In patients suffering from the progressive form of MS, the presence of HHV-6 does not "implicate the virus in the pathogenesis of the disease…and does not necessarily mean that HHV-6 was present at the time of diagnosis or that its presence was more than a chance event" (Voumvourakis et. al, 2010). Therefore, careful classification of patients relative to pattern and onset of MS would be beneficial in clarifying the connection between HHV-6 and MS.

The third condition described was "laboratory diagnostic methods must be objectively evaluated and harmonized" (Voumvourakis, Kitsos, Tsiodras, Petrikkos, & Stamboulis, 2010). It would be helpful if diagnostic methods could be both valid and objective. This would result in enhancement of the understanding of pathogens in diseases such as MS. Further, it is absolutely essential that both positive and negative results be included to "rule out the possibility of a group of negative controls resulting from technical errors in the experimental process" (Voumvourakis et. al, 2010). The researchers also discussed the limitations of their study, which included the vast amount of different

techniques used in each study, the areas of the brain examined, and that tissue collection was based mainly on availability instead of careful match between the patient and control group.

Treatment and Future Directions

Due to the lack of an effective vaccine, the development of new, tolerable and safe drugs is necessary for treatment of HHV-6. At the moment, treatment of HHV-6 is administered through the usage of conventional anti-herpes drugs such as ganciclovir (GCV) or valganciclovir (ValGCV), which is often accompanied by adverse side effects. In this context, researchers considered "the antivial activities reported for the sesuiterpene lactone arteminsinin and its derivative artesunate" (Millbradt, Auerochs, Korn, & Marschall, 2009). Artesunate (ART) is a semi-synthetic derivative of artemisinin and is frequently used to treat severe cases of malaria. Previous meta-analysis of malaria patients treated with artmenisinin and its derivatives establishes that this drug is indeed safe (Adjuik et. al, 2004). The antiviral capabilities of ART have been seen against human cytomegalovirus, herpes simplex virus and other herpesviruses (Millbradt et. al, 2009). Because of its abundant bioavailability and negligible side effects, ART is an ideal candidate for treating HHV-6.

To test HHV-6 sensitivity to ART, Millbradt et. al (2009) first cultured human cells that were successfully infected with HHV-6 and then treated the cultures with ART to measure the inhibition of viral protein synthesis (Western blot analysis) or viral genome replication (qPCR) and used immunofluorescence or plaque reduction assays to determine IC_{50} levels. Results were positive and ART was effective against HHV-6 by diminishing early

and late viral protein synthesis as well as reducing HHV-6A genome replication (Millbradt et. al, 2009). Thus, ART induces anti-HHV6-6 activity *in vitro* and may be useful in treatment of HHV-6 infections.

There has also been recent research on type 1 interferons as a possible way to treat HHV-6A. In this study type 1 IFN (α/β) was used to attempt to control the HHV-6 infection. It was found that the laboratory strains and isolates of HHV-B were resistant to type 1 IFN (α/β) due to "antiviral action as a result of improper IFN-stimulated gene (ISGs)" (Jaworska, Gravel, & Flamand, 2010). They found that HHV-6A, however, infected cells were sensitive to type 1 IFN (α/β) and show exceptional antiviral activity. This may be due to the divergence in the intermediate-early 1 (IE1) protein between the two variants. Between the two HHV-6 variants the IE1 protein varies between 62 and 71% in amino acid identities (Jaworska et. al, 2010). While the exact role of IE1 protein is unknown, it was recently shown that it is effective at obstructing IFN-β gene transcription, suggesting that it is involved in initial infection and latency (Jaworska, Gravel, Fink, Grandvaux, & Flamand, 2007).

In HHV-6B the IE1 protein "interacts with STAT2 and sequestrates it to the nucleus" (Jaworska et. al, 2010). As a result, the IE1B protein blocks the binding of ISGF3 to IFN-responsive gene promoters and this causes ISG silencing. In HHV-6A, however, the IE1 protein displayed "marginal ISG inhibitory activity relative to HHV-6B" (Jaworska et. al, 2010). It was found that the ISG inhibitory region was in a 41 amino acid sequence that was present in IE1B, but absent in IE1A. If the IE1B region was transferred it resulted in a gain of function that gave inhibitory ISG activity to IE1A. Jaworska et. al (2010) concluded that this demonstrates that "type 1 IFN signaling defects in HHV-6B-infected cells and highlights a major biological

difference between the HHV-6 variants" (Jaworska et. al, 2010). The information gained in this study contributes to what we know about how the virus functions.

Another possible way to treat HHV-6 could be to interfere with HHV-6 DNA replication. DNA replication in HHV-6 is catalyzed by the viral encoded DNA polymerase pU38 and the processivity factor pU27, which stabilizes pU38 during replication. Researchers studied the genetic polymorphism of pU27 seen in 46 clinical strains of HHV-6 A and B as well as four other strains that are antiviral resistant. They discovered that there are "28 amino acid changes (7.6%) and a two-amino acid deletions amongst the 368 residues of pU27, when using the U1102 (variant A) sequence as a reference" (Bonnafous et. al, 2010). In addition, they found that there was a median intravariant amino acid variability of 1.2% and 0.3% for A and B, respectively (Bonnafous et. al, 2010). Since pU27 is highly conserved and vital in viral replication, it may be a possible target for antiviral chemotherapy.

Conclusion

Human herpesvirus 6 is rapidly becoming an emerging virus of concern and in a modern day where there is an increased amount of immunocompromised people it is becoming more and more relevant. This can be accounted for by the swell in the usage of immunosuppressive drugs and the spread of AIDs. In this context, it is important that we research and develop safe treatments for HHV-6 that do not have severe side effects like the antivirals that have been traditionally used. HHV-6 is also highly significant because the serological prevalence amongst any given population is quite high. In addition, HHV-6's role in MS, a debilitating disease whose mechanisms and causes are still relatively

unknown, is promising, but requires more research. If the scientific community devotes more time and effort to understanding the viral etiology and mechanisms of HHV-6, the development of safer and more efficient treatments and even a vaccine will become a reality.

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