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Viruses in Chronic Progressive Neurologic Disease

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Abstract

Viruses have long been implicated as triggers of disease onset and progression in multiple sclerosis (MS) and similar neuroinflammatory disorders. Decades of epidemiological, molecular and pathologic studies have most strongly linked the human herpesviruses Epstein Barr virus (EBV) and human herpesvirus 6 (HHV-6) with MS. However, these viruses are ubiquitous in the general population and typically acquired decades before disease presentation, complicating the study of how they might contribute to disease. As experimental animal models may help elucidate mechanisms that have linked viruses with MS, we have been studying HHV-6 infections in small nonhuman primate. We recently demonstrated that the subsequent induction of an MS-like experimental neuroinflammatory disease results in significantly accelerated disease in HHV-6 inoculated marmosets compared to controls. Ultimately, disease intervention in the form of clinical trials with an anti-viral agent is the best way to concretely demonstrate a role for HHV-6 or any other virus in MS.

Keywords

Human herpesvirus 6 (HHV-6); herpesviruses; viral triggers; Multiple Sclerosis; CNS inflammation

Viruses in MS

Multiple sclerosis is an inflammatory disease affecting the central nervous system (CNS), resulting in tissue damage that often leads to neurologic dysfunction. MS is multifactorial in nature and thought to develop in genetically susceptible individuals following exposure to certain environmental factors. Infections, particularly viral infections, have long been linked to both disease onset and clinical progression. The idea of an infectious etiology originated with Charcot when MS was first being described as a clinical entity. For the past approximately 60 years, there have been many reports of agents detected in MS patient CSF and tissue that led to claims of ‘the MS pathogen.’ However, the field has largely transitioned away from searching for ‘the pathogen’ towards investigating the role of agents that are ubiquitous in the general population [1]. Two viruses that today remain the most strongly associated with MS are the herpesviruses Epstein Barr virus (EBV) and human herpesvirus 6 (HHV-6).

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Compelling evidence that herpesviruses contribute to MS pathology stems from the observation of herpesvirus-specific oligoclonal bands in MS CSF. OCBs are immunoglobulins to specific antigens, and intrathecal production reflects a compartmentalized CNS immune response, which is highly associated with CNS infections. Several years ago, Virtanen and colleagues demonstrated that in a cohort of MS patients, 38% exhibited CSF IgG OCB specific for either HHV-6 or EBV [2]. Oligoclonal bands are commonly observed in CNS disorders with an infectious etiology, and when the inciting agent is known, the bands are often specific to that agent. The late neurologist Dr. Don Gilden wrote in a 2001 JAMA editorial that "if EBV or any other virus causes MS, it should be possible to demonstrate that MS oligoclonal bands contain antibody directed against the suspected agent" [3]. Therefore, the identification of HHV-6- and EBV-specific bands in a subset of MS patients has strengthened the idea that these viruses might be directly involved in the pathogenesis of MS.

An introduction to human herpesvirus 6 (HHV-6)

Primary infection with HHV-6 occurs in most individuals within the first few years of life and transmission is thought to occur primarily via saliva within families. The nasopharynx and olfactory pathway are thought to be natural routes of entry for many herpesviruses, including HHV-6 [4]. A 2005 study of 130 children demonstrated primary HHV-6 infection in 40% by 12 months of age, and in 77% by 24 months of age. Fever is the most common finding in children with HHV-6 primary infection [5].

HHV-6, along with another closely related beta herpesvirus HHV-7, are known as roseolaviruses because upon primary infection, roseola occurs in approximately 20% of febrile children. Roseola is a self-limiting exanthematous disease characterized by a rash that usually begins on the trunk and spreads to the back, legs and neck, and lasts approximately 1–2 days [6]. Seizures occur in a small subset of children who present with febrile symptoms. It is hypothesized that childhood febrile seizures lead to an increased risk of hippocampal sclerosis, which leads to an increased risk of mesial temporal sclerosis and temporal lobe epilepsy. There is currently a prospective, multicenter study of the long-term outcomes of prolonged febrile seizures in childhood, with the hypothesis of HHV-6B involvement [7].

There are two species of HHV-6, HHV-6A and HHV-6B, which share about 95% overall nucleotide identity. Some open reading frames however, have less than 70% identity [8], which likely accounts for the differential tropisms, drug susceptibilities and disease associations that have led to their recent reclassification as distinct viruses [9]. Though the viral genome usually persists as an extrachromosomal episome, HHV-6 can also integrate into host cell chromosomes; chromosomally integrated HHV-6 is estimated at a prevalence of 0.6–1% in the general population [10].

While HHV-6 often persists asymptomatically in immunocompetent individuals, it can cause severe disease upon reactivation in the context of immunosuppression [11]. In fact, HHV-6 was originally isolated from PBMC cultures from patients with AIDS-associated lymphoproliferative disorders. HHV-6 reactivation occurs in nearly 50% of all bone marrow

and 20–30% of all solid organ transplant recipients within several weeks post-transplant and is also common among patients undergoing allogeneic or autologous hematopoietic stem cell transplants (HSCT) [12]. The majority of post-transplant reactivation is reported to occur with HHV- 6B. Viral reactivation can result in clinical symptoms ranging from fever and skin rash to bone marrow suppression and encephalitis [11].

The association of HHV-6 with Multiple Sclerosis

Neurotropism and perhaps even a CNS reservoir for HHV-6 has been suggested based on studies reporting viral DNA in the brains and CSF of MS patients and controls. Concomitant studies reported higher levels of HHV-6 expression in MS brains compared to control brains, and greater levels of viral DNA and viral mRNA specifically in the demyelinated plaques. The observations of viral mRNA and protein expression in oligodendrocytes underscored the hypothesis of HHV-6 as a driver of MS pathology (reviewed in [13]). Collectively, these studies demonstrated that while HHV-6 may be commensal in the normal brain, its replication and activity is enriched in the context of MS pathology. However, not all published studies agree with the conclusion of higher HHV-6 viral loads in MS versus control brains, or in MS lesions versus normal appearing white matter areas. There are myriad technical reasons why some but not all studies will have positive findings; it is necessary to keep in mind that the absence of evidence is not in itself evidence of absence.

In addition to studies of the virus in CNS tissues, many studies have linked the detection of HHV-6, or an immune response to HHV-6, in the periphery of MS patients with clinically active disease and these observations appear valid across geographically varied populations. Multiple studies report increased levels of HHV-6 antibodies in MS cohorts compared to controls. A 2012 study of a Tasmanian cohort found HHV-6 IgG titer to be a significant predictor of relapse risk and a 2014 study of a Spanish cohort found that decreased HHV-6 antibody titers correlated with fewer relapses and less disease progression (reviewed in [13]).

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 and remitting phases, significantly higher titers were detected during relapses compared to remissions [14]. Elevated antibodies against a latency-promoting protein may be one mechanism leading to the increased viral levels observed across many MS cohorts, as several studies report an increase in serum viral DNA during relapses [15, 16].

Demonstrating an association between a ubiquitous agent and a clinical disorder

Despite compelling human molecular and pathological studies that have associated HHV-6 with several CNS disorders, it has been difficult to prove causation in clinical disease. This is due to several factors, including i) the ubiquity of HHV-6 in the general population; ii)

observational studies often yield associative results that cannot demonstrate causation; and iii) there is no well-established animal model of HHV-6.

Interventional studies such as clinical trials are effective for demonstrating the involvement of an agent in a disorder, as the agent can be targeted and specific outcomes monitored and correlated with measurable clinical changes. However, to date there is no effective, specific anti-HHV-6 intervention to employ in clinical trials of patients with MS or other HHV-6 associated CNS disorders.

In the absence of interventional studies, experimental studies to assess outcomes of infection present an alternative. Humans are the only known hosts of HHV-6, and the lack of an animal model has impeded mechanistic studies of viral pathogenesis, drug susceptibility and proof of concept disease association studies. While not extensive, there have been recent efforts to investigate HHV-6 infection in various animal models, including transgenic and humanized mice and several non-human primate species [17].

Modeling HHV-6 as a trigger of neuroinflammatory disease

Animal models of HHV-6 infection have been difficult to establish because rodents lack the HHV-6 receptor for cellular entry, the complement regulatory receptor CD46 [18]. The common marmoset (*Callithrix jacchus)* is a small New World non-human primate that naturally expresses CD46, and is therefore susceptible to infection with HHV-6. Human and marmoset CD46 are conserved at the nucleotide level, supporting a rationale for experimental inoculations with HHV-6.

Marmosets are ideal models for studying the pathogenesis and host response to a human virus due to their genetic and immunologic proximity to humans, in addition to their broad behavioral range [19]. Marmosets have been infected with other human herpesviruses including VZV [20], Kaposi's sarcoma-associated herpesvirus (KSHV) [21], as well as non-herpesviruses such as dengue (DENV) [22]. Moreover, marmosets are especially appropriate for the study of a virus like HHV-6, implicated in neurologic conditions, due to their lissencephalic brain, sharing similar anatomic organization and grey to white matter volume ratio as humans [19, 23].

Our laboratory has published work examining primary infections of HHV-6A and HHV-6B in the marmoset [24]. In our first study, we reported different biological responses to HHV-6A and HHV-6B, now classified as separate viral species [9]. Moreover, we demonstrated differences in immunologic and virologic outcomes when the same virus was administered intravenously or intranasally [24].

As HHV-6 is almost ubiquitously acquired in early childhood, it is difficult to determine its role in a disease like MS that often manifests in early adulthood. Therefore, in a more recent study, we examined the effects of HHV-6 on the subsequent development of an experimental neuroinflammatory disease, experimental autoimmune encephalomyelitis (EAE). EAE is a widely-studied model of MS, in which animals are sensitized with white matter antigens to result in autoimmune inflammatory-mediated CNS demyelination. Marmoset EAE presents with more radiologic and pathologic similarities to MS compared to rodent EAE [25], and is

considered to represent a preclinical translational bridge for developing MS therapeutics [26].

In this study, we administered HHV-6A or HHV-6B intranasally and several months later induced EAE. Given the hypotheses of viral involvement in MS, we hypothesized that virus-inoculated marmosets would exhibit a more aggressive EAE course compared to controls (manuscript in preparation).

HHV-6 inoculated marmosets remained asymptomatic throughout the period of viral inoculations, during which a subset mounted antiviral antibody responses and had detectable viral DNA in blood and saliva. Viral antigen was detected, though infrequently, in the brain of an intranasally inoculated marmoset. These data are reminiscent of findings in humans, in which infections are often acquired asymptomatically and the virus persists at low, but at times detectable, levels in blood, saliva and CNS tissue.

Following EAE induction, the HHV-6 inoculated marmosets exhibited significantly accelerated clinical EAE compared to the uninfected control marmosets, with earlier symptom onset and reduced survival following the appearance of brain lesions. HHV-6 viral antigen was found to be upregulated in EAE brain lesions, which is similar to observations of enriched viral nucleic acids and antigens in MS lesions (reviewed in [13]). In the marmoset study, HHV-6 antigen was found to colocalize with T cells. In humans, HHV-6 antigen has been found to colocalize with oligodendrocytes, lymphocytes and glial cells [27, 28]. The virus inoculated but not control marmosets exhibited a peripheral expansion of interferon gamma-producing effector memory CD8 T cells that correlated with time post-EAE induction. These data suggest that administering HHV-6 intranasally may have primed the differentiation of this proinflammatory subset and contributed to the accelerated EAE in these animals (manuscript in preparation).

Viral infections can set the stage for neuroinflammation

The marmoset data summarized above suggest that viruses like HHV-6 can prime the immune system and subsequently lead to the exacerbation of inflammatory-mediated disorders, including neuroinflammation. This is reminiscent of a hypothesis developed by Fujinami and colleagues termed the fertile field hypothesis [29]. This hypothesis weaves together the MS susceptibility factors of genetics (particularly MHC alleles) and viral infections and importantly, contextualizes viral infections as contributing factors rather than etiologic agents.

The fertile field hypothesis puts forth that infections can induce a heightened immunologic state, which in the presence of additional antigens (environmental, viral, or self), can lead to the expansion of autoreactive T cells. This expanded pool of autoreactive T cells can then develop into autoaggressive cells in the presence of additional antigens (environmental, viral, or self). This concept incorporates the idea of a threshold for autoimmunity, which is set in part by an individual’s genetics, and regulated by the quality and magnitude of infections and the host immune responses to those infections [29].

Clinical trials to validate the hypothesis of viral involvement in MS

Ultimately, the only way to convincingly demonstrate the involvement of any exogenous agent in a disease is through controlled clinical trials using prophylactic or therapeutic means to specifically interfere with that agent. In neurologic disease, CNS penetrability is an important consideration. Currently, there is no HHV-6-specific antiviral drug or treatment, though the identification of such compounds, in addition to HHV-6 immunotherapy, remain active areas of study. Decades of work by our group and others provide rationale for the development of anti-HHV-6 interventions to be used in the setting of acute and chronic neuroinflammatory conditions like MS.

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