Department of Virology

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General Summary

Human herpesvirus (HHV) is capable of establishing a lifelong latent infection of their host, is reactivated frequently. We are studying the molecular mechanism of latency and pathogenesis of human cytomegalovirus (HCMV) and HHV-6, and find a novel latent protein of HHV-6 which associate with and mood disorders. We are also trying to apply HHV-6 and HHV-7 to the tools for studying the mechanism of fatigue. Salivary amounts of HHV-6 and HHV-7 DNA increased with training and decreased with rest, suggesting the usefulness of viral DNA as biomarkers of physiological fatigue and cancer-related fatigue.

Research Activities

Posttranscriptional regulation of HHV-6 immediate-early 1 and 2 genes by immediate-early 2 and splicing factor squamous-cell carcinoma antigen recognized by T cells 3
Background: Herpesviruses operate a gene regulatory cascade conventionally divided into immediate-early (IE), early, and late phases. However, this cascade is not so simple in β-herpesviruses, such as HCMV, HHV-6A, and HHV-6B, and posttranscriptional regulation has been suggested to play an important role in the replication of these viruses.
Methods: To determine the effects of IE1B/IE2B (derived from HHV-6B [strain HST]) on the alternative splicing of ie1A/ie2A messenger (m) RNA expressed from a cosmid (Cosmid SalI derived from HHV-6A [strain U1102]), ie1A/ie2A expression was examined with the reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR), using primer pairs that can discriminate HHV-6A from HHV-6B.
In addition, to reveal the mechanism underlying HHV-6B IE2B posttranscriptional regulation, an HHV-6B (strain HST)-infected peripheral blood mononuclear cell complementary DNA library was screened by the yeast two-hybrid method, with IE2B as the bait. Furthermore, the correlation between the expression levels of sart3 and ie1B/ie2B mRNA in phytohemagglutinin-stimulated peripheral blood mononuclear cells or cord blood mononuclear cells infected with HHV-6B (strain HST) was examined by RT-qPCR 24 hours after infection.
Results and conclusion: In this study, we demonstrated that the alternative splicing of ie2 mRNA is enhanced by IE2 itself through an interaction with the protein squamous-cell carcinoma antigen recognized by T cells 3 (SART3), which is known to be involved in pre-mRNA processing. In addition, we showed that sart3 mRNA expression correlates with ie1/ie2 mRNA expression in peripheral blood mononuclear cells and umbilical cord blood mononuclear cells infected with HHV-6B. These results suggest that the interaction between IE2 and SART3 plays a significant role in the posttranscriptional regulation of ie1/ie2 and that SART3 is a key cellular factor that determines viral replication and prolif-

eration during postentry stages.

Attenuation of HHV-6B reactivation by aging
Objective: There has been little research on HHV-6B in healthy adults, and prevalences in different age groups have been unclear. Therefore, this study evaluated seroprevalence to HHV-6 antibodies in ordinary working people and examined the effect of aging on seroprevalence. Because HHV-6B is reactivated in saliva, this study also investigated an association between age and HHV-6B reactivation based on measured salivary HHV-6 DNA levels.
Methods: The subjects were 77 ordinary office workers who underwent a health checkup. In this population, we measured anti-HHV-6 antibody titers using enzyme-linked immunosorbent assay and salivary HHV-6 DNA levels. In addition to examining an association with age, we examined associations with body mass index, smoking habit, and alcohol consumption as confounding factors.
Results: The seropositivity of HHV-6 antibodies decreased significantly in subjects 50 years and older, and age was significantly negatively correlated with anti-HHV-6 antibody titers. Age and salivary HHV-6 DNA levels were also significantly negatively correlated but were not significantly correlated with other factors.
Conclusion: Our results suggest that HHV-6B reactivation is attenuated by aging. Thus, HHV-6 antibodies steadily decrease in the body with aging.

Increased levels of interleukin 1β and basic fibroblast growth factor in cerebrospinal fluid during HHV-6B encephalitis
A member of the β herpesvirus subfamily, HHV-6 is further subdivided into HHV-6A and HHV-6B. Exanthema subitum typically results in fever and rash but resolves spontaneously without further complications or illness. However, in rare cases, HHV-6B infection can lead to encephalitis and has major clinical implications. Immunodeficiency associated with clinical procedures, such as hematopoietic stem cell transplantation, has been reported as a factor in HHV-6B-induced encephalitis; however, in cases of primary HHV-6B infection without immunodeficiency, the factors responsible for disease onset remain elusive. We detected higher levels of interleukin (IL)-1β and basic fibroblast growth factor (bFGF) in the cerebrospinal fluid of patients with HHV-6B encephalitis when compared to those in patients with non-HHV-6B-induced febrile seizures. *In vitro*, IL-1β and bFGF enhanced HHV-6B gene expression in infected U373 astrocytes during the initial and maintenance phases of infection, respectively. These findings indicate that IL-1β and bFGF contribute to HHV-6B growth and the onset of encephalitis.

Publications

Kobayashi N, Nishiyama T, Yamauchi T, Shimada K, Suka M, Kondo K, Yanagisawa H. Attenuation of human herpesvirus 6B reactivation by aging. *J Med Virol.* 2019 Jul; **91**(7): 1335-1341. doi: 10.1002/jmv.25434. Epub 2019 Feb 27. PubMed PMID: 30788852.