Department of Virology

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General Summary

Human herpesvirus (HHV) 6, which can establish lifelong latent infections of hosts, is frequently reactivated. We are studying the molecular mechanisms of the latency and pathogenesis of HHV-6. Additionally, we are using HHV-6 and HHV-7 to study the mechanism of fatigue and as viral vectors for gene therapy.
Fatigue is an indispensable biological "alarm" for avoiding the state of exhaustion that is caused by severe stress and overwork and might also induce a variety of diseases. We have investigated the molecular mechanisms of the reactivation of HHV-6 and HHV-7, which are stimulated by fatigue, and identified the molecule that can induce viral reactivation during fatigue.
Using our understanding of HHV reactivation, we have developed a method for measuring the accumulation of fatigue by determining the amounts of HHV-6 and HHV-7, which are reactivated and released into the saliva.

Research Activities

Assessment of work-related long-term fatigue and differentiation from chronic fatigue syndrome by using salivary HHV-6 and HHV-7 reactivation as a biomarker
Fatigue is composed of physical weakness, brought about by stress and other factors, and feelings of fatigue, such as exhaustion and tiredness. Long-term fatigue can be caused by work-related chronic stress, whereas chronic fatigue syndrome (CFS) can be triggered by an infection that results in feelings of fatigue that continue for a long period. These conditions can lead to a reduction in manpower and other social problems. To date, no effective and objective method has been developed to assess long-term fatigue. Moreover, long-term fatigue has been difficult to distinguish from CFS, which is also characterized by feelings of fatigue.
To develop an objective method of quantifying work-related long-term fatigue, we investigated the use of the HHV-6 and HHV-7, which are reactivated by fatigue or stress. The results showed an increase in salivary HHV-6 DNA copy numbers which correlated with the number of hours of office work. Research on Japanese Self-Defense Forces personnel whose workload was fully controlled showed that an increase in the amount of training produced a reversible increase in salivary HHV-6 and HHV-7 DNA copy numbers.
An investigation, in an animal model, of the molecular mechanism of HHV reactivation by fatigue showed an increase in inflammatory cytokines, a phenomenon already thought to play a part in the molecular mechanism of fatigue. The results also demonstrated a relationship between fatigue and the novel phenomenon of the induction of differentiation markers in myeloid cells, which are latent infection sites for this group of viruses.

Furthermore, in patients with CFS, we observed almost no increase in salivary HHV-6 and HHV-7 DNA copy numbers, demonstrating that fatigue in CFS and long-term work-related fatigue have different characteristics. These results suggest that salivary HHV-6 and HHV-7 reactivation, which is a simple and objective biomarker of long-term fatigue, might also help increase our understanding of the molecular mechanism of fatigue and improve the diagnosis of CFS.

Novel gene therapy viral vector using nononcogenic lymphotropic herpesvirus

Despite the use of retroviral vectors, efficiently introducing target genes into immunocytes, such as T cells, is difficult. In addition, retroviral vectors carry risks associated with the oncogenicity of the native virus and the potential for introducing malignancy in recipients due to genetic carryover from immortalized cells used during vector production. To address these issues, we have established a new virus vector that is based on HHV-6, a nononcogenic lymphotropic HHV that infects CD4+ T cells, macrophages, and dendritic cells. In the present study, we altered the cell specificity of the resulting recombinant HHV-6 by knocking out the U2-U8 genes. The resulting virus proliferated only in activated cord blood cells and not in peripheral blood cells. Umbilical cord blood cells produced replication-defective recombinant virus in a sufficiently high titer to omit the use of immortalized cells during vector production. The HHV-6 vectors led to high rates (>90%) of gene transduction in both CD4+ and CD8+ T cells. These viruses showed low-level replication of viral DNA that supported greater expression of the induced genes than that of other methods but that was insufficient to support the production of replication-competent virus. Furthermore, HHV-6 vectors containing short hairpin RNAs against CD4 and human immunodeficiency virus (HIV) Gag markedly inhibited the production of these proteins and of HIV particles. Our results demonstrate the utility of HHV-6 as a new noncarcinogenic viral vector for treating immunologic diseases and for immunotherapy.

Identification of SITH-1 as novel latent protein of HHV-6 associated with CFS and mood disorders

HHV-6 has exhibited the most promise as a candidate CFS-associated virus. We identified a novel HHV-6 latent transcript that was expressed during the relatively activated latent stage (intermediate stage) of HHV-6 latency. This transcript encoded the small open reading frame named small protein encoded by the intermediate transcript of HHV-6 (SITH) 1. In the present study we aimed to identify SITH-1 responsible for CFS. In addition, to determine the function of SITH-1 in the brain, we analyzed the behavior of mice that expressed SITH-1 in the brain.

We have studied the expression of SITH-1 by examining the prevalence of anti-SITH-1 antibodies in persons with CFS or mood disorders and in healthy persons. Antibody detection was by indirect immunofluorescence and enzyme-linked immunosorbent assay. Next, an open reading frame of SITH-1 was linked downstream of a glial fibrillary acidic protein promoter, and expressed in glial cells of mice using an adenovirus vector. After growth, the mice were analyzed with the tail suspension test, prepulse inhibi-

tion, and locomotor activity.
With an indirect fluorescent antibody method, the rate of SITH-1 positivity was high in patients with CFS or mood disorders. In addition, enzyme-linked immunosorbent assay showed a high correlation. In behavioral experiments, 3-week-old SITH-1 mice showed decreased immobility time in the tail suspension test and impaired prepulse inhibition. Meanwhile, 5-week-old SITH-1 mice showed a decrease in spontaneous motor activity and an increase in immobility time in the tail suspension test. Therefore, astrocytes exposed to SITH-1 seem to play a major role in depressive and manic-like behavior of mice. These results suggested that SITH-1 is involved in the onset of mood disorders.