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General Summary

Human herpesviruses (HHVs) are capable of establishing lifelong latent infections of their hosts and are reactivated frequently. We are studying the molecular mechanism of latency and the pathogenesis of HHV-6 and searching for latent proteins of HHV-6 associated with chronic fatigue syndrome (CFS) and mood disorders. Additionally we are attempting to apply HHV-6 and HHV-7 as tools to study the mechanism of fatigue and as viral vectors for gene therapy. Furthermore, we studied the relationship between herpes simplex virus (HSV) reactivation and Alzheimer's disease (AD).

Research Activities

Increase in the immunoglobulin G avidity index due to HSV type 1 reactivation and its relationship with cognitive function in amnestic mild cognitive impairment and AD

After infection with HSV type 1 (HSV-1), latent infection persists for life in the trigeminal ganglion, and reactivation results in an outbreak of cold sores around the mouth. Many previous studies have reported HSV-1 reactivation to be a risk factor for AD. This study enrolled subjects with AD (n = 85); subjects with amnestic mild cognitive impairment (aMCI), a prodromal stage of AD (n = 34); and healthy control subjects (n = 28). The avidity index of anti-HSV-1 immunoglobulin (Ig) G antibodies—a known indicator of HSV-1 reactivation—was measured to clarify the relationship between HSV-1 reactivation and symptoms of cognitive function in AD.

Cognitive function in AD and aMCI was evaluated with scores from the Mini-Mental State Examination and the Frontal Assessment Battery. The results showed that the subjects with aMCI, in whom cerebral function is better preserved than in subjects with AD, had a higher anti-HSV-1 IgG antibody avidity index than did subjects with AD or healthy controls. Furthermore, the anti-HSV-1 IgG antibody avidity index was even higher in the subjects with high Mini-Mental State Examination scores on orientation to time and 3-step command subscores. We observed a negative correlation between the anti-HSV-1 IgG antibody avidity index and the plasma concentration of brain-derived neurotrophic factor, which is an indicator of encephalitis. This finding suggests that HSV-1 reactivation, as observed through an increase in the anti-HSV-1 IgG avidity index, does not progress to encephalitis.

These results suggest that HSV-1 reactivation occurs from the stage of aMCI, which is prodromal to AD, and can affect AD symptoms without an intermediary stage of severe encephalitis. The study suggests that the anti-HSV-1 IgG antibody avidity index is a useful biomarker for the early diagnosis of aMCI or AD and that antiviral medication to treat HSV-1 could play a role in preventing the onset of AD.

Assessment of work-related long-term fatigue and differentiation from CFS 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 CFS can be triggered by an infection that results in chronic feelings of fatigue. 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. We found that an increase in salivary HHV-6 DNA copy numbers correlated with the number of hours of office work. Research on Japanese Self-Defense Force personnel, whose workload is 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 role in the molecular mechanism of fatigue. We also found 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 is a simple and objective biomarker for long-term fatigue and may also help further our understanding of the molecular mechanism of fatigue and improve the diagnosis of CFS.

Novel gene therapy viral vector using nononcogenic lymphotropic HHV

Gene introduction into T cells is a useful technique for gene therapy of human immunodeficiency virus (HIV) infection and the immunotherapy of fatal diseases, including cancer. This method now relies on vectors derived from members of the lentivirus family of retroviruses to introduce genes into T cells. A major advantage of retroviral vectors is the high efficiency with which they introduce genes into target cells. However, the pathogenicity of the native virus has long caused unease regarding the use of viral vectors. In particular, oncogenicity is a characteristic of wild-type retroviruses; another risk factor is the potential recombination of retroviral vectors with endogenous retroviruses in the host to yield replication-competent virus.

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 umbilical cord blood cells but not in peripheral blood cells. Umbilical cord blood cells produced replication-defective recombinant virus in a sufficiently high titer to make unnecessary 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 expression of the induced genes that was greater than that of other methods but was insufficient to support the production of replication-competent virus. Furthermore, HHV-6 vectors containing short hairpin RNAs against CD4 and 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 determine whether SITH-1 is 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 inhibition, 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.

Publications

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