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General Summary

The human beta-herpesvirus subfamily consists of human cytomegalovirus (HCMV), human herpesvirus (HHV)-6, and HHV-7. Both HHV-6 and HHV-7 belong to the Roseolovirus genus of the β-herpesviruses, and the HHV-6 species are divided into two variants: HHV-6A and HHV-6B. The β-herpesviruses can establish a lifelong latent infection of the host and are frequently reactivated; some evidence suggests that the molecular mechanisms of viral latency and reactivation are shared by these viruses. We are studying the molecular mechanism of latency and pathogenesis of β-herpesviruses. Additionally, we are studying these viruses to investigate the mechanisms of fatigue and to develop viral vectors for gene therapy.

Research Activities

Chronic fatigue syndrome and herpesvirus

Chronic fatigue syndrome (CFS) is a disease of unknown etiology in which the main complaint is severe fatigue. The prominence of the acute onset of illness, the persistent symptoms consistent with a viral infection, and the increased titers of viral antibodies and enhanced activity of the interferon-induced enzyme suggest viruses play a role in CFS. In other words, CFS may be a kind of "postinfectious fatigue syndrome" following any viral infection. A variety of viruses have been evaluated, including enteroviruses, retroviruses, and human herpesviruses.

CFS is a condition that lasts far longer than postinfectious fatigue. For this reason, the infection causing CFS is believed to be a latent infection with a herpesvirus. The most promising candidate as a CFS-associated virus is HHV-6. Because an unusual latent infection with HHV-6 which may be a cause of CFS, studies of latent infection are considered important for elucidating CFS.

Several lines of evidence suggest that latent HHV-6 infection in the brain may be involved in development of some neurological conditions, such as recurrent febrile convulsion, multiple sclerosis, and encephalitis. However, the site of HHV-6 latency in the brain has not been identified.

To determine how CFS develops, we attempted to identify a special latent HHV-6 infection state termed the "intermediate phase." In this phase, several kinds of HHV-6 latency-associated proteins corresponding to Epstein-Barr virus nuclear antigens might be expressed. This intermediate stage is observed in the first phase in which HHV-6 commences reactivation but differs from reactivation in that no virus is produced. To examine the relationship between latent-infection proteins, whose expression is enhanced in the intermediate stage, and disease, serum titers of antibodies to cells that express

latent-infection proteins at high levels were examined in patients with CFS. This examination revealed that 40% of patients with CFS showed antibody reactions to intermediate-phase latent HHV-6 infection, whereas healthy subjects showed virtually no reaction.

Beta-herpesvirus latency and reactivation

Fatigue is a common problem of modern life. Many people who are under considerable stress have various kinds of fatigue. However, few scientific and medical studies have examined fatigue, and even fewer studies have involved definitive means or quantitative standards for quantitatively and objectively measuring fatigue, which is subjective.

Muscle fatigue (exercise fatigue) has mainly been studied as a representative example of fatigue. The indicator examined in most studies is the increase in the production of lactic acid in muscles. However, lactic acid is an important energy source for the central nervous system, and the theory that it inhibits muscle activity has been questioned. In addition, during muscle fatigue pyruvic acid levels increase and pH decreases in body fluids. These phenomena are indeed observed in response to the stress of a muscle load (exercise load); however, fatigue is distinct from local muscle exhaustion and is considered to be a broader and larger physiological phenomenon.

Both HHV-6 and HHV-7 are almost universally acquired by 2 to 3 years of age. These viruses belong to the beta-herpesvirus subfamily, and biological and molecular analyses show they are closely related to each other. Both viruses establish life-long latency, a hallmark of herpesviruses, reactivate frequently, and are shed in saliva.

To investigate viral reactivation, we have identified the latency-associated transcripts of HHV-6 and have clarified part of the mechanism of HHV-6 reactivation. HHV-6 establishes latency in macrophages, remains in a stable intermediate stage between latency and reactivation, and is reactivated by two or more factors.

HHV-6 can reactivate in immunosuppressed patients; however, the relationship between immunosuppression and the induction of reactivation is unclear. To identify the factor or factors involved in HHV-6 reactivation, we studied the association of HHV-6 reactivation and work-induced fatigue in healthy adults. Immune strength is thought to deteriorate when humans are fatigued, and virus infection is a possible manifestation of this deterioration. However, the relationship between fatigue and virus infection in humans remains unclear.

HHV-6 DNA was detected in 88% of subjects engaged in moderately excessive work due to long hours and other factors (the first test day). In contrast, HHV-6 DNA was detected in 23.8% of subjects immediately after a holiday (the second test day). These results show that HHV-6 is significantly reactivated on exertion. These results led to the discovery that HHV-6 DNA expressed in saliva through the reactivation of HHV-6 is a biomarker of the severity of fatigue. Accordingly, an simple, objective method for assessing the degree of fatigue by detecting HHV-6 DNA released into saliva as a result of the reactivation of HHV-6 was developed.

The amount of HHV-7 DNA was semiquantitatively measured with the double-nested polymerase chain reaction method in serially diluted saliva. HHV-7 DNA was detected

in 92% of patients with CFS. In contrast, HHV-7 DNA was detected in 50% of healthy subjects during work and in only 30% of healthy subjects at rest. The amount of HHV-7 DNA in half of patients with CFS was 10- to 100-fold higher than the average amount in healthy subjects. These results show that HHV-7 is significantly reactivated in the chronic fatigue state that accompanies disease. These results also led to the discovery that HHV-7 DNA expressed in saliva owing to reactivation of HHV-7 is a biomarker of fatigue which varies with the cause of chronic fatigue. Accordingly, an objective method for assessing the degree of fatigue by detecting HHV-7 DNA released into saliva owing to reactivation of HHV-7 was developed, enabling the simple assessment of the decline in physical strength caused by chronic fatigue.

Use of HHV-6 and HHV-7 as gene therapy vectors

Accumulation of knowledge and various technological advances in molecular biology and molecular genetics have contributed greatly to the recent progress in life science, providing abundant information about various biological phenomena. Active research and development is under way in various fields of life science, with particular interest in the analysis of gene functions. These activities have led to the development of techniques and vectors for introducing isolated genes into cells and individual living organisms.

Viral vectors have advantages over other known vectors in introducing a foreign gene into a cell for protein expression. The central idea underlying gene transfer with viral vectors is to introduce a foreign gene into an infected cell and transform the cell under the control of promoter sequences, taking advantage of the infectious capacity of the virus (productive infection, latent infection, abortive infection).

In particular, HHV-6 and HHV-7 have attracted considerable interest as candidate viral vectors for gene therapy, because infections with these viruses have mild symptoms. The use of herpesviruses, particularly HHV-6 and HHV-7, as recombinant viruses and as recombinant viral vectors has certain advantages, which include low pathogenicity, ease of gene introduction into blood cells, such as the T cells and macrophages, and introduction of relatively large genes. However, producing a recombinant virus or a recombinant virus vector that originates from HHV-6 or HHV-7 is difficult, and no method is available for producing such viruses and vectors. In addition to technical factors, the characteristics of the genes of HHV-6 and HHV-7 make recombination of HHV-6 and HHV-7 difficult.

We have identified the dispensable genes of HHV-6 and HHV-7 and have reported the establishment of recombinant HHV-6 and HHV-7. The dispensable locus of HHV-6 is 8.4 kbp, and that of HHV-7 is 7.3 kbp; both are useful sites for inserting a large gene. An exogenous nucleotide sequence may encode various substances, such as bacterial artificial chromosomes, cytokine genes, ribozyme, interference RNAs, immunological co-stimulator molecules, signal transduction molecules, enzymes, and chemical attractants. Furthermore, exogenous nucleotide sequences may be used for the gene therapy of mammals. Gene therapy might be used to prevent human immunodeficiency virus infection of compromised cells and for the immunotherapy of cancer.

Publications

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