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General Summary

Human herpesvirus (HHV) 6 belongs to the human β-herpesvirus subfamily, which consists of human cytomegalovirus, 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 2 variants: HHV-6A and HHV-6B. β-herpesviruses can establish a life-long latent infection of the host and are reactivated frequently, and some evidence suggests that the molecular mechanisms of viral latency and reactivation are common among these viruses. We are studying the molecular mechanisms of latency and pathogenesis of β-herpesviruses. Additionally, we are trying to apply these viruses to studies of the mechanism of fatigue and as viral vectors for gene therapy.

Research Activities

Mood disorders, chronic fatigue syndrome, and herpesvirus

Chronic fatigue syndrome (CFS) is a disease of unknown etiology whose chief complaint is severe fatigue. The prominence of the acute onset of illness, the persistent symptoms consistent with a viral infection, the increased titers of viral antibodies, and the enhanced activity of interferon-induced enzymes suggest viruses play a role in CFS. In other words, CFS might be a type of "postinfectious fatigue syndrome" following any viral infection. The viruses evaluated to date include enteroviruses, retroviruses, and HHVs.

CFS is a disease that lasts far longer than postinfectious fatigue. Therefore, the infection causing CFS might be a latent infection with an HHV. Of the HHVs, HHV-6 is the most promising candidate for a CFS-associated virus. Because an unusual latent infection with HHV-6 may cause CFS, the study of latent infection is important for determining the cause of CFS.

Several lines of evidence suggest that latent HHV-6 infection in the brain is involved in some neurological diseases, 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 the mechanism by which CFS is contracted, we attempted to identify a special latent HHV-6 infection state called the "intermediate phase," described above. In this phase, several types of HHV-6 latency-associated protein corresponding to EBNAs might be expressed. This intermediate stage is observed in the first phase when HHV-6 commences reactivation but is completely different from reactivation in that no virus is produced. To examine the relationship between disease and latent infection proteins, whose manifestation is promoted in the intermediate stage, titers of antibodies to cells in which latent infection proteins were well manifested were examined in the serum of patients with CFS. This examination revealed that about 40% of patients with

CFS showed antibody reactions to intermediate-phase HHV-6 latent infection, whereas healthy subjects showed virtually no reaction.

β-Herpesviruses latency/reactivation

Fatigue is a familiar problem of daily life. Many people who are under stress suffer from various kinds of fatigue. However, scientific and medical studies relating to fatigue have been inadequate, and few studies have employed clear, quantitative, and objective means for examining fatigue, which is subjective manifestation.

To date, muscle fatigue (exercise fatigue) has mainly been studied as a representative example of fatigue. The main indicator in muscle fatigue is an 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 lactic acid inhibits muscle activity has been disproven. In addition, during muscle fatigue, pyruvic acid increases and pH decreases in body fluids. These phenomena are indeed observed when a certain stress, i.e., a load to muscle (exercise load), is given; however, fatigue differs 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 β-herpesvirus subfamily and are closely related to each other, as shown by biological and molecular analyses. They 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, in part, the mechanism of HHV-6 reactivation. HHV-6 establishes latency in macrophages and maintains a fairly stable intermediate stage between latency and reactivation, which is induced by 2 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 of HHV-6 reactivation, we have studied the association with HHV-6 reactivation and work-induced fatigue in healthy adults. Immune strength is thought to deteriorate when humans are fatigued, and virus infection reflects this deterioration of immune strength. 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 working 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 fatigue biomarker (a biological index factor) that varies according to fatigue. Accordingly, a simple and objective method was developed for assessing the degree of fatigue by detecting HHV-6 DNA released into saliva as a result of the reactivation of HHV-6.

The amount of HHV-7 DNA was semiquantitatively determined with the double-nested polymerase chain reaction method after serial dilution of 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 the patients with CFS was 10 to 100 times the average amount in healthy persons. These results show that HHV-7 is significantly reactivated in the chronic fatigue state that accompanies disease. These results led to the discovery that HHV-7 DNA expressed in saliva due to reactivation of HHV-7 is a fatigue biomarker (a biological index) that varies with the severity of chronic fatigue caused by diseases or other factors. Accordingly, a simple and objective method was developed for assessing the degree of fatigue, and decline in physical strength caused by it, by detecting the HHV-7 DNA released into saliva due to reactivation of HHV-7.

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

Accumulation of knowledge and various technological advances in molecular biology and molecular genetics have greatly contributed to the recent progress in life sciences, providing much information about various biological phenomena. Research and development in various fields of life science are ongoing, with an emphasis on 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.

Virus 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 virus vectors is to introduce a foreign gene into an infected cell and transform the cell with the foreign gene under the control of promoter sequences, taking advantage of the infectious capacity of the virus (productive infection, latent infection, and abortive infection).

In particular, HHV-6 and HHV-7 have drawn much interest as candidate virus vectors for gene therapy, because infection with these viruses produces mild symptoms. Herpesvirus, and HHV-6 and HHV-7 in particular, have certain advantages as recombinant viruses and recombinant virus vectors, which include low pathogenicity, ease of gene introduction into blood cells, such as T cells and macrophages, and the ability to introduce large genes. However, no method has been available for producing recombinant viruses and recombinant virus vectors from HHV-6 or HHV-7. Factors complicating the recombination of HHV-6 and HHV-7, other than technical factors, are the characteristics of the HHV-6 and HHV-7 genes.

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 was approximately 8.4 kbp, and that of HHV-7 was approximately 7.3 kbp; these sites are useful for insertion of large genes. The exogenous nucleotide sequence may encode at least 1 type of substance, including artificial bacterial chromosomes, cytokine genes, ribozymes, interference RNA, 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 the infection of compromised cells by human immunodeficiency virus and for the immunotherapy of cancer.

Publications

Kondo K. Biology and application of herpesviruses for gene therapy. *Tanpakushitsu Kakusan Koso* 2007; **52**(10 Suppl): 1294–300.

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