Molecular and Virological Evidence From Viral Activation Chromosomally Integrated Human Herpesvirus 6A in a Patient With X-Linked Severe Combined Immunodeficiency

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(See the Editorial Commentary by Flamand on pages 549–51.)

It has been unclear whether chromosomally integrated human herpesvirus 6 (ciHHV-6) can be activated with pathogenic effects on the human body. We present molecular and virological evidence of ciHHV-6A activation in a patient with X-linked severe combined immunodeficiency. These findings have significant implications for the management of patients with ciHHV-6.

Keywords. ciHHV-6; HHV-6; X-SCID; hemophagocytic syndrome; thrombotic microangiopathy.

Human herpesvirus 6 (HHV-6) is a ubiquitous DNA virus that is the causative agent of roseola infantum, and infects individuals by 3 years of age [1]. After primary infection, HHV-6 establishes a latent state in the host. There are 2 distinct species, HHV-6A and HHV-6B. Most HHV-6 infections are caused by HHV-6B, whereas HHV-6A is less common. Chromosomally integrated HHV-6 (ciHHV-6) is the state in which HHV-6 (HHV-6A or HHV-6B) is integrated into the host germline genome, and it is transmitted vertically in a Mendelian manner. Although ciHHV-6 interacts about 1% of the general population, it is generally considered to be a nonpathogenic condition. However, it is unclear whether ciHHV-6 can be activated with pathogenic effects on the human body [2].

Severe combined immunodeficiency (SCID) is a group of genetic disorders that result in a combined absence of T- and B-cell immunity. It is characterized by life-threatening infections during the first year of life unless treated, usually with hematopoietic stem cell transplantation (HSCT). X-linked severe combined immunodeficiency (X-SCID) arises from a mutation in the interleukin 2 receptor, gamma (IL2RG) gene on the X-chromosome [3]. We encountered a boy with X-SCID in whom ciHHV-6A was activated.

CASE REPORT

A 2-month-old boy was hospitalized for recurrent episodes of fever, cough, diarrhea, and failure to thrive. Upon admission, a viral infection was suspected, and supportive care did not improve his symptoms.

Twenty days after admission, mild pancytopenia (leukocyte count, 1.4 × 10^9/L; hemoglobin level, 78 g/L; and platelet count, 37 × 10^9/L) and elevated aminotransferases and ferritin were evident (aspartate aminotransferase, 448 U/L; alanine aminotransferase, 448 U/L; alanine aminotransferase, 218 U/L; and ferritin, 4325 ng/mL) (Supplementary Figure 1). A bone marrow biopsy showed a hypocellular condition without dysplastic changes, as well as increased activated phagocytes. These results suggested hemophagocytic syndrome (HPS).

An immunological evaluation revealed an absence of T cells and low immunoglobulin levels. Genetic analysis identified a mutation in the IL2RG that was consistent with X-SCID. The patient’s mother was heterozygous for the same mutation, and there was no such mutation detected in the patient’s father.

A comprehensive search for a pathogen identified high levels of HHV-6 DNA (1.2 × 10^7 copies/µg DNA) in his peripheral blood. Antiviral treatment with ganciclovir or foscarnet did not reduce the viral load, and ciHHV-6 was suspected. We detected high levels of HHV-6 DNA in the patient’s fingernails, the father’s peripheral blood, and the father’s hair follicles (5.9 × 10^3, 1.0 × 10^7, 1.2 × 10^6 copies/µgDNA, respectively).
Fluorescence in situ hybridization analysis of the patient’s fibroblasts and his father’s peripheral blood mononuclear cells (PBMCs) confirmed HHV-6 integration at chromosome 22 in both individuals (Figure 1); these results suggested vertical germline transmission.

However, discontinuation of antiviral treatment led to a deterioration of the patient’s HPS. Because no other pathogen was detected, activation of HHV-6 was suspected. To confirm this suspicion, we performed 3 assays that could detect viral activation despite the presence of integrated HHV-6 DNA. First, reverse transcription polymerase chain reaction (RT-PCR) was used to detect viral RNA in whole-blood samples. RT-PCR was performed on 2 HHV-6 genes, the late gene U60/66 and the immediate-early (IE) gene IE1, as described previously [5]. We detected viral RNA for both genes (4.6 × 10^2 copies/µg RNA for U60/66 and 5.2 × 10^3 copies/µg RNA for IE1). Second, immunostaining was used to detect IE antigens in a bone marrow sample taken at the time of HPS (Figure 2 and Supplementary Figure 2) [6]. Last, HHV-6A was isolated from the patient’s PBMCs. It was cultured with cord blood cells and its presence confirmed by immunofluorescent staining with an anti–HHV-6 monoclonal antibody (Figure 3 and Supplementary Figure 3) [1].

Two hypotheses were postulated: Either the patient with ciHHV-6 was infected de novo with HHV-6, or HHV-6 was activated from the ciHHV-6 genome present in this patient. We performed a sequence analysis of the HHV-6 IE1 gene, as IE1 is variable and readily used to distinguish between HHV-6 variants [7]. DNA samples from isolated HHV-6A (described above), the patient’s fingernails, his father’s hair follicles, and laboratory strains U1102 and Z29 were amplified by PCR and sequenced. Because active HHV-6 is not present in the fingernails or hair follicles, we could amplify the original integrated HHV-6 strain from the genomes in these tissues. To our surprise, the sequences and subsequent phylogenetic analysis revealed that the isolated virus was identical to the original integrated HHV-6A strain present in both the patient and his father. Furthermore, this HHV-6A strain was unique in that it differed from all other HHV-6 strains analyzed (Supplementary Figure 4). These results suggested that the isolated HHV-6A strain originated from the activation of ciHHV-6A. Analysis of 3 other viral genes (gB, U94, and DR) confirmed these results [8, 9].

The resumption of antiviral drug treatment with prednisolone ameliorated the patient’s HPS. When he reached age 7 months, the patient underwent HSCT. Antiviral drug treatment was continued during HSCT, and engraftment was achieved 14 days after transplant. After engraftment, thrombotic microangiopathy (TMA) and gastrointestinal bleeding developed. Simultaneously, the patient’s HHV-6A DNA and RNA titers increased, and HHV-6A was reisolated. Anticoagulant therapy and a reduction in tacrolimus dosage gradually improved the patient’s TMA. With immunological reconstruction, the patient’s HHV-6A DNA and RNA titers were successfully reduced and ultimately, no HHV-6A was isolated from subsequent blood samples. The asymptomatic patient was discharged at 12 months.

**DISCUSSION**

Since the discovery of ciHHV-6 in 1993, the question of whether ciHHV-6 can be activated from its integrated state has been perpetually debated [2]. With this case report, we provide the first molecular and virological evidence of viral activation from ciHHV-6A in the human body. This evidence comprises (1) viral RNA and antigens detected in PBMCs and bone marrow, as well as HHV-6A isolated from PBMCs; (2) HHV-6A sequences integrated into the patient’s and his father’s genomes, which were identical to those of the isolated virus; and (3) antiviral treatment and immunological reconstruction, which were effective in treating this activated ciHHV-6A.

In an effort to understand the biological significance of ciHHV-6, active viral replication from ciHHV-6 has recently been demonstrated in vitro under specific experimental conditions [9–11]. However, only a few studies have suggested ciHHV-6 activation in vivo despite high ciHHV-6 prevalence (approximately 1%) in the general population [12–14]. Activation of ciHHV-6 in vivo has been previously reported in mothers with ciHHV-6 who passed on the infection to infants who did not have inherited ciHHV-6 [8]. Our findings are consistent with these findings, as we clearly demonstrate the activation of HHV-6A in a patient who acquired ciHHV-6 via germline transmission.

![Figure 1](image_url)

**Figure 1.** Integration of human herpesvirus type 6 (HHV-6) in chromosome 22 was demonstrated by fluorescence in situ hybridization analysis. Fibroblasts derived from the patient’s skin (A) and peripheral blood mononuclear cells from the father (B) were cohybridized with HHV-6-specific (yellow arrow) and chromosome-22-specific probes (white arrows) [4]. HHV-6 integration in only one of the chromosome 22 alleles was shown in both materials. In sets of A and B are the enlarged images of FISH data positively cohybridized with both probes.
We speculate that the presence of X-SCID allowed for efficient activation of ciHHV-6A, and this phenomenon was detected with several technical strategies. Similarly, RT-PCR and virus isolation showed conversion from an HHV-6–positive status to a negative status with the patient’s immunological recovery. In addition, these techniques were used to test samples taken from the patient’s father. We were able to determine that he was indeed the ciHHV-6 carrier, yet he was HHV-6A negative. This suggests that X-SCID influenced the activation of ciHHV-6A. Because X-SCID prevalence is extremely low (about 0.001%), this case provides valuable insight into immunocompromised individuals and HHV-6 infection. However, the mechanism that triggered ciHHV-6A activation and replication in this patient remains to be elucidated. Further studies of patients with ciHHV-6 are required to determine what causes activation of this latent integrated virus.

The association between HHV-6 and HPS has previously been reported [15], and a link between HHV-6 and TMA has also been noted [16]. Therefore, it is possible that ciHHV-6A activation in our patient was associated with HPS and TMA. We noted that active HHV-6A infection coincided with symptom onset and the active infection was controlled with antiviral treatment. This suggests that HHV-6A is pathogenic, yet it remains to be established whether activated HHV-6A enhances underlying pathological conditions, and whether the activation of ciHHV-6A occurs in a similar fashion for all infected individuals.

Latent HHV-6 reactivation occurs in 40%–50% of recipients during HSCT, and our case report is the first to demonstrate that ciHHV-6A activation also occurs during this procedure. It is possible that the presence of X-SCID allowed for viral activation, but further studies are required to validate this hypothesis.

We have described the first case to provide molecular and virological evidence of the activation of chromosomally integrated HHV-6A in the human body. However, our report has limitations. We still do not know how virus production was triggered from a state of ciHHV-6A or how the production of the virus affected the patient’s symptoms. Despite these limitations, based on this case, we hypothesize that an immunodeficient phenotype in conjunction with uncontrolled host defense systems allows the activation of ciHHV-6A. We support the recommendation that a screening program to detect ciHHV-6 in

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**Figure 2.** Histology and human herpesvirus type 6 (HHV-6) immunostaining. A, Hematoxylin and eosin staining of bone marrow. B and C, Immunostaining with an anti–HHV-6 antibody.

**Figure 3.** Immunofluorescent staining assay. A, Virus isolation confirmed with an anti–human herpesvirus type 6 antibody (gp116/64/54). B, U1102 cultured with cord blood cells (positive control). C, Cord blood cells alone (negative control).
transplant patients and donors be established, and recommend that ciHHV-6 patients with immunocompromised status such as primary immunodeficiency, human immunodeficiency virus infection, or organ transplantation, be monitored carefully.

**Supplementary Data**

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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