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CASE REPORT

Persistent Long-Term Human Herpesvirus 6 (HHV-6) Infection in a Patient with Langerhans Cell Histiocytosis

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Langerhans cell histiocytosis (eosinophilic granuloma) was first diagnosed in the adolescence of a male patient presented. Several years later persisting human herpesvirus 6 (HHV-6) infection was recognized. The HHV-6 infection could be verified retrospectively in his historical histological samples; the continuous presence of HHV-6 could be established through 17 years of disease course. The patient was operated several times during this period for painful relapses, and developed diabetes insipidus. At variable time points during the clinical course, Varicella zoster (VZV), Epstein-Barr virus (EBV) and human herpesvirus 8 (HHV-8) infections were temporarily detected from blood samples and biopsy specimens. HHV-6 was the only virus continuously identified throughout the entire follow-up period. Antiviral therapy effectively cleared EBV and HHV-8, but HHV-6 remained detectable throughout the disease course. Since DNA sequences of HHV-6 could be detected in the pathologic histiocytes of eosinophilic granuloma, and from other samples taken later on, it is suggested that long-term HHV-6 infection may be associated with development or progression of Langerhans cell histiocytosis. (Pathology Oncology Research Vol 13, No 2, 157–160)

Key words: Langerhans cell histiocytosis, eosinophilic granuloma, HHV-6, human herpesviruses, herpesvirus reactivation

Introduction

Langerhans cell histiocytosis (LCH), also specified as histiocytosis X, eosinophilic granuloma, Letter-Siwe syndrome and Hand-Schüller-Christian disease, is characterized by skin rash, dyspnea, diabetes insipidus, bone pain, weight loss, fever, gingival hypertrophy, balance and memory disorders. Irradiation of involved organs, corticosteroids and chemotherapy can be applied in multifocal diseases. There is consensus on treatment, however, controlled virological studies have not been performed. Human herpesvirus 6 (HHV-6) belongs to the group of Betaherpesvirinae together with cytomegalovirus (CMV) and human herpesvirus 7 (HHV-7). The HHV-6 has variants A and B. Variant HHV-6A may cause roseola, but the majority of asymptomatic infections are followed by persistent viremia. HHV-6B is well-known for causing the majority of *exanthema subitum* cases, a childhood disease. After primary infection, the virus may also persist in the body, causing latent infection, but fever, diarrhea, rush, neurological symptoms and hepatitis are caused occasionally as well. Following transplantation, reactivation of HHV-6 may occur with encephalopathy, pneumonitis, delayed platelet engraftment or thrombotic microangiopathy. HHV-6 had been found to replicate in different types of cells,[1,4,5,8,10,15] and has been shown to be associated with LCH.[6,9]

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Materials and methods

Blood plasma, buffy coat and bone marrow samples were taken from the patient and stored at -20°C. Paraffin block samples of surgically removed granulomas were also avail-

able. Simple and nested PCR methods were used for the detection of the presence of lymphotropic herpesviruses.[2,3,12-14] The routine histological examination and specific immunohistochemical staining (CD1a, S100) demonstrated the characteristic histiocytes of LCH. *In situ* hybridization with alkaline phosphatase reaction was used for the detection of viral DNA.

Clinical history of the patient and results

The history of the Caucasian male patient (born in 1975) was as follows. Continuous HHV-6 viremia could be detected in his samples taken from the diagnosis of the disease throughout 17 years of its course (and ongoing), using simple and nested PCR. He presented with right facial pain twice after the age of 11. Maxillary sinus drainage had to be done because of inflammation.

A right cranial tumor developed at the age of 14, the surgical removal also involved the orbit. The histological diagnosis was Langerhans cell histiocytosis. Remission was seen after postoperative irradiation (*Fig. 1*).

At the age of 19, a circumscribed eosinophilic granuloma was surgically removed from the iliac bone, but no further treatment was required.

Several bones became involved at the age of 22 (one lesion in the right iliac bone, two lesions in the left femur). At this stage, the underlining disease and diabetes insipidus were also confirmed. Occipital zoster-like skin-lesions appeared subsequently and its etiology was confirmed by serology.

In the following two years no further progression occurred.

His state deteriorated at the age of 25, viremia caused by 3 different lymphotropic herpesviruses could be detected simultaneously (EBV, HHV-6 HHV-8). As a result of famcyclovir (3 weeks) and interferon-á treatments (6 months), both EBV and HHV-8 disappeared from the blood. The HHV-6 viremia remained persistent, however, a zoster-like lesion appeared once again on his nape.

At the age of 26, a new painful lesion appeared in the right humerus. The progression could be stopped by 3 cycles of etoposide + methylprednisolone treatment, followed by interferon-á maintenance treatment for 6 months.

A new painful relapse occurred on his skull at the age of 30. Surgical removal and monthly zoledronate infusions proved to be effective again.

The patient is currently symptom-free, in good condition. He is the father of two children.

Histological samples removed in 1989 and 1994 have been shown to be positive for the presence of HHV-6 DNA while they were negative for EBV and HHV-8 (Vysis, Amersham). *Fig. 2* shows the image of the histological examination of tumor tissue sample taken in 1989. The

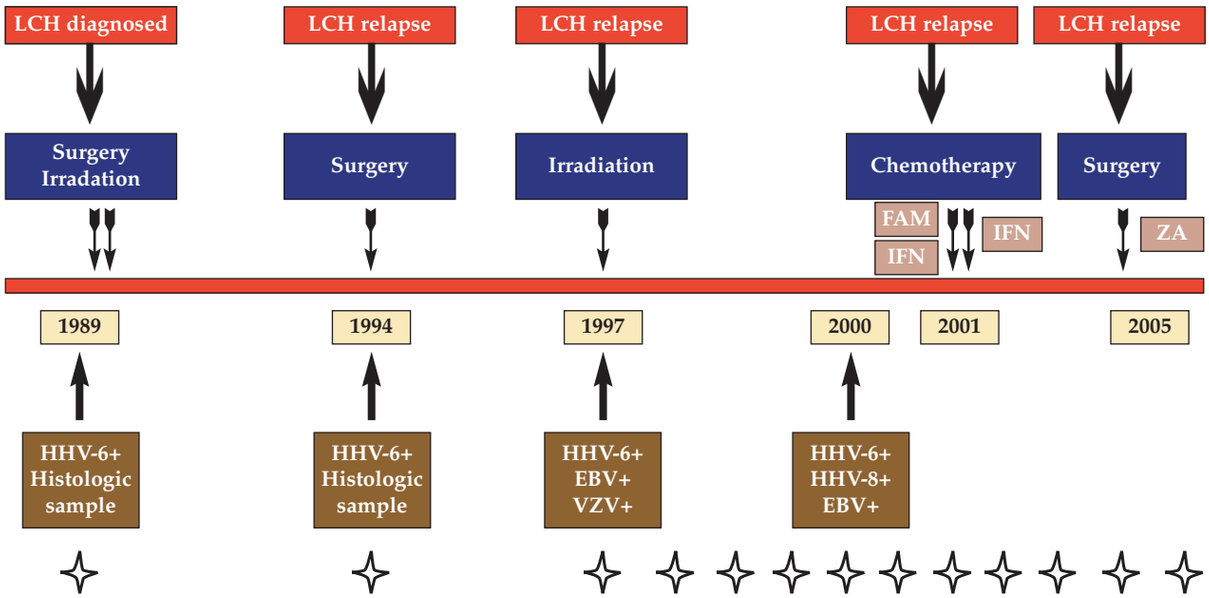

Figure 1. *Schematic representation of the disease course, therapeutic interventions and virology results of the case patient. Major therapeutic interventions (upper row). Clinical relapses are shown by thick vertical arrows plotted above the time scale. The type of chemotherapy is shown between the thick arrows. Time points for proven HHV-6 DNA positivity in blood, bone marrow or histological samples are shown by 4-armed asterisks in the lowest row of the figure. Historical samples and detection of multiple herpesviruses from the same samplings are boxed. FAM: Famcyclovir treatment, IFN: alpha-interferon treatment, ZA: monthly zoledronate infusions, LCH: Langerhans cell histiocytosis*

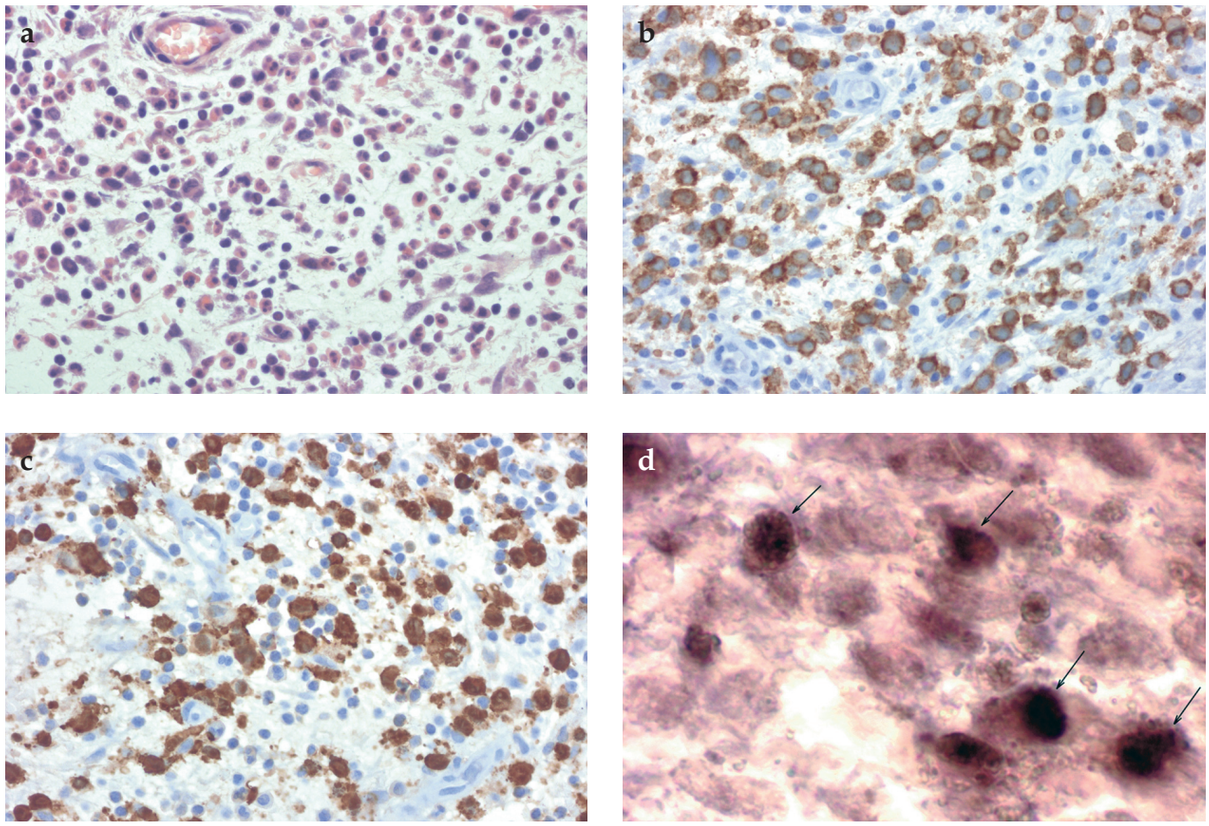

Figure 2. *Histology of Langerhans cell histiocytosis and HHV-6 in situ hybridization. A representative section of the tumor tissue obtained in 1989, stained with hematoxylin-eosin, depicts the characteristic histiocytes of LCH* ***(a).*** *Positive reactions in the tumor cells with CD1a* ***(b)*** *and S100* ***(c)*** *immunostaining. In situ hybridization for HHV-6 in the tumor tissue from the same material* ***(d).*** *Purple-red staining of the alkaline phosphatase reaction (arrows) indicates the presence of HHV-6 DNA in the nuclei of characteristic histiocytes. (Magnification: a-c: 400x, d: 1000x)*

characteristic Langerhans histiocytes can be recognized by CD1a and S100 immunoreaction. A tissue sample was processed for HHV-6 detection by *in situ* hybridization. As shown in *Fig. 2d*, HHV-6 viral DNA was detected in the histiocytes characteristic of LCH, while the rest of the sample shows less intense (background) staining.

Discussion

The possible involvement of HHV-6 and HHV-8 infections in LCH has been suggested previously, however, follow-up studies failed to further substantiate this observation.[6,9,11] Glotzbecker et al. conducted a study on LCH biopsies, in which they documented that their prevalence in LCH patients is the same as found in patients without the disease.[7] Since HHV-6 seropositivity can be demonstrated in >95% of the adult population, an innocent bystander role for this virus cannot be ruled out even in our case reported. Long-term presence of HHV-6 in immune-competent patients was shown to last even for a decade.[16] Our patient exhibits the longest persistence of HHV-6 (17 years) published to our knowledge. HHV-8 was absent from the histological preparations, confirming findings of Jenson et al.[9] The long-term presence of HHV-6 could be the consequence of immune-modulation caused by LCH, however, no obvious signs of immunodeficiency were seen, except for the two Varicella zoster reactivations. Our case may suggest a potential association between HHV-6, other human herpesviruses and the progression of LCH. Our observation may also support the need for further investigation of HHV-6 and other herpesviruses in a cohort of several patients with Langerhans cell histiocytosis.

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