HHV-6 Positive Reed-Sternberg Cells in Nodular Sclerosis Hodgkin Lymphoma

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ABSTRACT

Classical Hodgkin lymphoma (HL) is comprised of malignant Reed-Sternberg (RS) cells scattered within a mixed inflammatory background. The unusual bimodal age distribution of HL suggests that infectious agents may play a role in etiology. The presence of Epstein-Barr virus (EBV) within the RS cells in a proportion of HL cases supports this idea. However, the most common subset of HL, nodular sclerosis HL (NSHL), is the subset least often EBV-associated. HHV-6 is a near-ubiquitous herpesvirus that acquired in childhood and most often followed by asymptomatic lifelong persistence. Since a few reports have suggested a role for HHV-6 in HL, we have analyzed a cohort of NSHL cases for both EBV and HHV-6, and sought to specifically localize these viruses to the malignant RS cells. Formalin-fixed paraffin-embedded lymph nodes from 20 patients with newly diagnosed nodular sclerosis Hodgkin lymphoma were analyzed for HHV-6. HHV-6 FISH, two non-overlapping HHV-6 DNA PCR products. EBV, HHV-6, and HHV-6A and HHV-6B sequences were amplified by PCR. Southern blotting of genomic DNA and in situ hybridization using both dsDNA and RNA probes confirmed the presence of abundant HHV-6 DNA within cells from Hodgkin lymphoma. HHV-6 DNA was detected in lesions from 13 of 17 cases of NSHL by HHV-6-ISH PCR. These results were confirmed by Southern blotting. HHV-6 protein was detected in the majority of cases by IHC. In some cases positive staining was detected only in small lacelike areas. HHV-6 positivity was detected in RS cells from 12 of 20 cases (Figure 1). In no case was HHV-6 found exclusively within RS cells, and only infrequently was HHV-6 detected in the majority of RS cells. In some cases with HHV-6 positive RS cells, DNA was isolated by laser capture micro-dissection (LCM) from HHV-6-positive RS cells and subjected to HHV-6 PCR. In comparison with the HHV-6 viral load from whole tissue DNA, the viral load from LCM-enriched RS cells was significantly higher. This result indicates that HHV-6 in these cases is preferentially located within RS cells. In contrast to these HHV-6 results, the distantly related herpesvirus EBV was detected by EBER ISH within RS cells in only 5 of 20 NSHL cases. Three cases contained RS cells that are positive for both EBV and HHV-6. Although not statistically significant, dual positivity of RS cells trends toward older age, while HHV-6 positivity alone trends toward younger age. HHV-6 FISH confirmed the presence of abundant HHV-6 DNA within cells from Hodgkin lymphoma. In conclusion, HHV-6 DNA is frequently detected within RS cells of NSHL cases. Further studies to examine the contribution of HHV-6 to the etiology of HL are ongoing.

RESULTS

HHV-6 DNA was detected in lesions from 13 of 17 cases of NSHL by HHV-6-ISH PCR. These results were confirmed by Southern blotting. HHV-6 protein was detected in the majority of cases by IHC. In some cases positive staining was detected only in small lacelike areas. HHV-6 positivity was detected in RS cells from 12 of 20 cases (Figure 1). In no case was HHV-6 found exclusively within RS cells, and only infrequently was HHV-6 detected in the majority of RS cells. In some cases with HHV-6 positive RS cells, DNA was isolated by laser capture micro-dissection (LCM) from HHV-6-positive RS cells and subjected to HHV-6 PCR. In comparison with the HHV-6 viral load from whole tissue DNA, the viral load from LCM-enriched RS cells was significantly higher. This result indicates that HHV-6 in these cases is preferentially located within RS cells. In contrast to these HHV-6 results, the distantly related herpesvirus EBV was detected by EBER ISH within RS cells in only 5 of 20 NSHL cases. Three cases contained RS cells that are positive for both EBV and HHV-6. Although not statistically significant, dual positivity of RS cells trends toward older age, while HHV-6 positivity alone trends toward younger age. HHV-6 FISH confirmed the presence of abundant HHV-6 DNA within cells from Hodgkin lymphoma. In conclusion, HHV-6 DNA is frequently detected within RS cells of NSHL cases. Further studies to examine the contribution of HHV-6 to the etiology of HL are ongoing.

INTRODUCTION

Hodgkin lymphoma is one of the most frequently occurring lymphomas in the Western world. The classical variant of Hodgkin lymphoma is characterized by malignant Reed-Sternberg (RS) cells scattered within a mixed inflammatory background of lymphocytes, eosinophils, and neutrophils. The unusual bimodal age distribution suggests that infectious agents may play a role in the etiology. The well-documented presence of Epstein-Barr virus (EBV) within the RS cells in a proportion of cases supports this idea. However, the most common subset of HL, nodular sclerosis HL (NSHL), is the subtype least often EBV-associated. HHV-6 is a near-ubiquitous herpesvirus that acquired in childhood and most often followed by asymptomatic lifelong persistence. We have analyzed the lymph node tissues from patients with NSHL for both EBV and HHV-6, and sought to specifically localize these viruses to the RS cells.

MATERIALS & METHODS

Formalin-fixed paraffin-embedded lymph nodes from 20 patients with newly diagnosed nodular sclerosis Hodgkin lymphoma were examined. For each case EBER ISH (Ventana, HHV-6 IHC (HHV6(IgG)), Santa Cruz, see Figure A), and HHV-6 PCR was performed protocol. In cases with HHV-6 positive RS cells by IHC, laser capture micro-dissection (LCM, Leica) was performed to enrich for RS cells. DNA from LCM-enriched RS cells was subjected to qPCR using primers specific to HHV-6 DNA. Southern blotting of genomic DNA and in situ hybridization using both dsDNA and RNA probes confirmed the presence of abundant HHV-6 DNA within cells from Hodgkin lymphoma. In conclusion, HHV-6 DNA is frequently detected within RS cells of NSHL cases. Further studies to examine the contribution of HHV-6 to the etiology of HL are ongoing.

CONCLUSIONS

By detecting the presence of HHV-6 in cases of nodular sclerosis Hodgkin lymphoma by immunohistochemistry, PCR (Southern blot), and FISH, our current results extend earlier findings by other investigators suggesting an association between HHV-6 and Hodgkin lymphoma (see references table). Given that we find HHV-6 within RS cells, HHV-6, like EBV, may play a role in the pathogenesis of some cases of Hodgkin lymphoma.

REFERENCES