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

Alexa J. Siddon, MD[1], Larissa Lozovatsky[1], Ayman Mohamed[2], and S. David Hudnall, MD[1]

Department of [1]Pathology, Yale School of Medicine, and [2]Precipio Diagnostics, New Haven, CT, USA

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 subtype of HL, nodular sclerosis HL (NSHL), is the subtype least often EBV-associated.

HHV-6 is a near-ubiquitous herpesvirus first acquired in childhood characterized most often by asymptomatic life-long 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 primary cases of NSHL were examined by EBER ISH, HHV-6 IHC, and HHV-6 PCR followed by Southern blot. In cases with HHV-6 positive RS cells by IHC, laser capture microdissection (LCM) was performed to collect purified RS cells. DNA from LCM-captured RS cells was extracted, amplified by whole genome amplication, and subjected to HHV-6 PCR to confirm that the RS cells were HHV-6 positive.

Of 17 cases of NSHL, 13 were HHV-6 PCR positive (76%). Five of 20 cases (25%) contained numerous EBV positive RS cells and 10 of 21 cases (48%) contained numerous HHV-6 positive RS cells by IHC (in 3 cases RS cells were positive for both HHV-6 and EBV). The presence of HHV-6 specifically within RS cells was confirmed both by HHV-6 PCR on LCM-captured RS cells and by HHV-6 FISH.

We have demonstrated that HHV-6 genome is present within the neoplastic RS cells of a significant proportion of NSHL cases, most of which were EBER ISH negative. These findings support that in some cases, HHV-6 may play a role in the etiology of NSHL. 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 agent(s) 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, most cases (>60%) of the nodular sclerosis subtype of HL (NSHL) are EBV negative. HHV-6 is a near-ubiquitous herpesvirus first acquired in childhood and and most often followed by asymptomatic life-long persistence. We have analyzed the lymph nodes from patients with NSHL for both EBV and HHV-6, and sought to specifically localize virus 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(20), Santa Cruz, see **Figure A**), and HHV-6 U94 PCR was performed per protocol. In cases with HHV-6 positive RS cells by IHC, laser capture microdissection (LCM, Leitz) was performed to enrich for RS cells. LCM-derived DNA was subjected to whole genome amplification (Genome Plex Reamplification Kit, Sigma) to yield sufficient DNA for multiple PCR assays. Positive PCR results were confirmed by sequencing of PCR products.

For HHV-6 FISH, two non-overlapping HHV-6B DNA fragments (27 and 38 kb) isolated from cosmid clones (pMF147-31 and pMF210-8, HHV-6 Foundation, Santa Barbara, CA) were directly labeled with Alexa Fluor 488 according to manufacturer instructions (FISH Tag DNA Green Kit, Invitrogen). Controls consisted of formalin-fixed paraffin-embedded pellets of HHV-6 infected and uninfected cell lines HSB-2 and MOLT-3 (HHV-6 Foundation, Santa Barbara, CA).

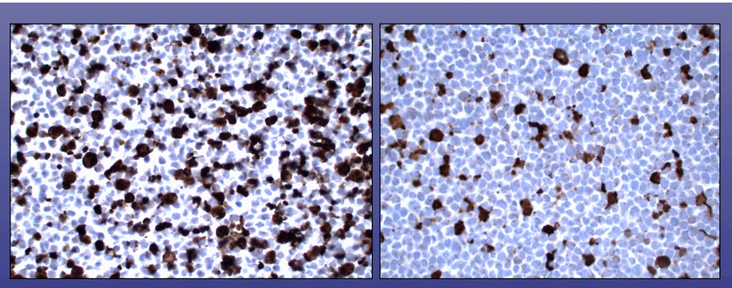

Figure A. HHV-6 immunohistochemistry. Left panel = HSB-2 cell line infected with strain A HHV-6 (late culture). Right panel = MOLT3 cell line infected with strain B HHV-6 (early culture).

RESULTS

HHV-6 DNA was detected in lesional tissue from 13 of 17 cases of NSHL by HHV-6 U94 PCR. These results were confirmed by Southern blotting.

HHV-6 protein was detected in the majority of cases by IHC. While in some cases positive staining was detected only in small leukocytes **(Figure 1)**, HHV-6 positivity was detected in RS cells from 10 of 21 cases **(Figure 2)**. 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) of CD30-positive cells and subjected to U94 PCR **(Figure 3)**. 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 of 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 **(Figure 4)**.

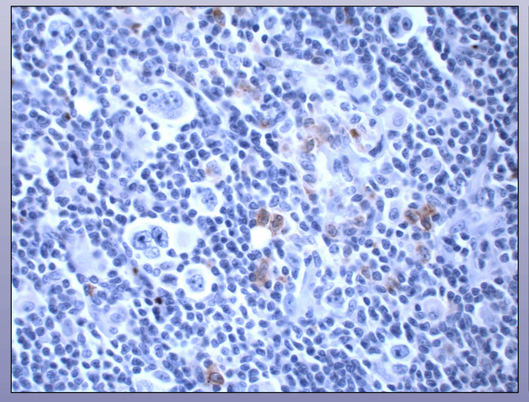

Figure 1. Immunohistochemical staining for HHV-6 in a case of NS Hodgkin lymphoma. In this case, the RS cells are negative while scattered small cells are positive.

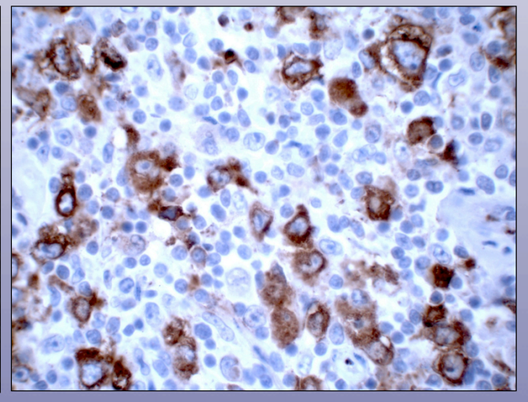

Figure 2. Immunohistochemical staining for HHV-6 in a case of NS Hodgkin lymphoma. In this case, the RS cells are positive.

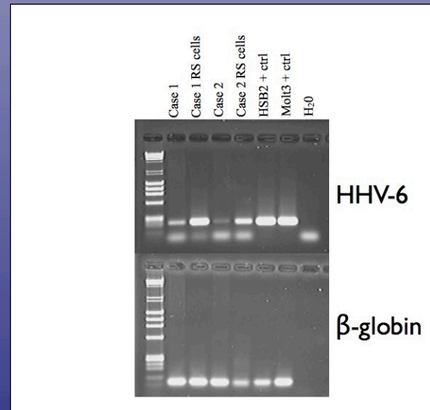

Figure 3. HHV-6 U94 PCR detection of viral DNA within lymph nodes and an elevated level within isolated RS cells. HHV-6 positive cell lines serve as a positive control.

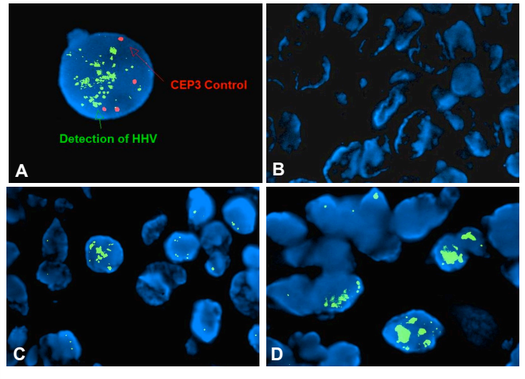

Figure 4. HHV-6 FISH. **A.** HHV-6B MOLT3 infected cell line. **B.** HHV-6 negative tissue control (tonsil). **C.** HHV-6 positivity in HHV-6+ HL tissue (case 1). **D.** HHV-6 positivity in HHV-6+ HL tissue (case 2).

TABLE 1: Twenty-one Cases of HL Were Examined for EBV, HHV-6 IHC, and HHV-6 PCR

NSHL	Age & Sex	Site of disease	EBER ISH (RSC)	U94 PCR (Southern blot)	HHV-6 IHC (RSC)
1 - 2598	54 M	inguinal	POS	POS	POS
2 - 3881	23 M	mediastinal	neg	POS	neg
3 - 4154	22 F	mediastinal	neg	POS	neg
4 - 4393	17 F	cervical	neg	neg	POS
5 - 8536	17F	cervical	neg	POS	POS
6 - 8981	12 M	mediastinal	neg	neg	neg
7 - 10395	14 F	mediastinal	neg	nd	neg
8 - 12430	16 M	cervical	neg	POS	POS
9 - 13322	31 M	cervical	POS	POS	neg
10 - 14079	66 M	inguinal	nd	nd	POS
11 - 14764	13 F	cervical	neg	POS	POS
12 - 15215	38 M	axillary	neg	POS	neg
13 - 16703	60 M	subcarinal	POS	POS	POS
14 - 18060	64 M	inguinal	neg	nd	neg
15 - 18116	16 F	cervical	neg	POS	neg
16 - 18951	20 M	cervical	POS	neg	neg
17 - 19020	39 M	cervical	neg	POS	neg
18 - 20257	70 M	inguinal	POS	neg	POS
19 - 20430	14 M	cervical	neg	POS	neg
20 - 20526	15 M	cervical	neg	POS	POS
21 - 28864	16 M	cervical	neg	nd	POS
	age range 12-70 median age 20 M:F 2.5:1		5/20+ (25%)	13/17+ (76%)	10/21+ (48%)

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 in Hodgkin lymphoma (see references below).

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

1. Marasca R, Luppi M, Montorsi M, et al. *[Identification of human herpesvirus HHV-6 sequence in a case of Hodgkin's disease by polymerase chain reaction]. Medicina* (Firenze). 1990; 10(1):43-45.
2. Torelli G, Marasca R, Luppi M, et al. *Human herpesvirus-6 in human lymphomas: identification of specific sequences in Hodgkin's lymphomas by polymerase chain reaction.* Blood. 1991; 77(10):2251-2258.
3. Krueger GR, Guenther A, Knuefermann R, et al. *Human herpesvirus-6 (HHV-6) in Hodgkin's disease: cellular expression of viral antigens as compared to oncogenes met and fes, tumor suppressor gene product p53, and interleukins 2 and 6.* In Vivo. 1994;8(4): 501-516.
4. Rojo J, Ferrer Argote VE, Klueppelberg U, et al. *Semi-quantitative in situ hybridization and immunohistology for antigen expression of human herpesvirus-6 in various lymphoproliferative diseases*. In Vivo. 1994; 8(4):517-526.
5. Luppi M, Barozzi P, Garber R, et al. *Expression of human herpesvirus-6 antigens in benign and malignant lymphoproliferative diseases*. Am J Pathol. 1998; 153(3):815-823.
6. Schmidt CA, Oettle H, Peng R, et al. *Presence of human beta- and gamma-herpes virus DNA in Hodgkin's disease*. Leuk Res. 2000; 24(10):865-870.
7. Krueger GR, Huetter ML, Rojo J, Romero M, Cruz-Ortiz H. *Human herpesviruses HHV-4 (EBV) and HHV-6 in Hodgkin's and Kikuchi's diseases and their relation to proliferation and apoptosis*. Anticancer Res. 2001; 21(3C):2155-2161.
8. Lacroix A, et al. *HHV-6 and EBV DNA quantitation in lymph nodes of 86 patients with Hodgkin's lymphoma*. J Med Virol. 2007; 79:1349-1356.
9. Lacroix A, et al. *Involvement of human herpesvirus-6 variant B in classic Hodgkin's lymphoma via DR7 oncoprotein*. Clin Cancer Res. 2010; 16:4711-4721.