Human *Herpesvirus* 8–Associated Intravascular Lymphomatosis Within an AIDS Patient

JD Henning[1], LD Presser[2], SM Kulich[3,4], MA Nalesnik[3] and Frank J Jenkins[2,3]

[1]Department of Biology, University of Pittsburgh at Johnstown, Johnstown, PA, USA. [2]Department of Infectious Diseases and Microbiology, University of Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA, USA. [3]Department of Pathology, University of Pittsburgh, Pittsburgh, PA, USA. [4]Department of Pathology, VA Pittsburgh Healthcare System, Pittsburgh, PA, USA.

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ABSTRACT: Human herpesvirus 8 (HHV-8) causes Kaposi sarcoma (KS) and is associated with B-cell malignancies. Intravascular large B-cell lymphoma is an uncommon B-cell lymphoma characterized by proliferation of neoplastic B cells within the lumina of blood vessels. Although the underlying cause of this lymphoma is unclear, a previous report has identified a single case of HHV-8–associated intravascular lymphomatosis (IVL). We analyzed 5 cases of IVL for the presence of HHV-8, finding the second case of HHV-8–associated IVL. Human herpesvirus 8 protein expression (latency-associated nuclear antigen 1 [LANA-1]), Epstein-Barr virus–encoded RNAs (EBERs), and the cellular markers CD20 and CD45 were determined by immunohistochemistry. Intravascular lymphoma cells from a subject with human immunodeficiency virus (HIV) were positive for HHV-8 LANA-1, EBV EBER, and CD20 and CD45 expression. The 4 non-HIV–infected IVL cases were negative for HHV-8 LANA-1 and EBER expression. Future studies are required to determine whether the association between HHV-8 and HIV with IVL is true.

KEYWORDS: HHV-8, intravascular lymphomatosis, HIV

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CORRESPONDING AUTHOR: Frank J Jenkins, Department of Infectious Diseases and Microbiology, University of Pittsburgh Cancer Institute, University of Pittsburgh, G.16 Hillman Cancer Research Pavilion, 5117 Centre Avenue, Pittsburgh, PA 15213, USA. Email: fjenkins@pitt.edu

Introduction

Human herpesvirus 8 (HHV-8) is the causative agent of Kaposi sarcoma (KS), rare primary effusion lymphoma, and some forms of multicentric Castleman disease.[1] Human herpesvirus 8 is also known as KS-associated herpesvirus. Serological studies determine the prevalence rate in the United States to be between 3% and 10%, whereas the seroprevalence of HHV-8 is higher from endemic areas such as southern Italy (35%) and Africa (over 50%).[2-6] Transmission of HHV-8 can occur through both sexual and nonsexual routes.[7-9]

Intravascular lymphomatosis (IVL) is a proliferation of neoplastic mononuclear cells within the lumina of blood vessels.[10,11] It is described as an extranodal variant of non-Hodgkin lymphoma and in 2001 classified as a subtype of diffuse large B-cell lymphoma by the World Health Organization Classification.[12] Intravascular lymphomatosis occurs most often in B cells, but rare cases in T cells and natural killer (NK) cells have also been reported.[13] Epstein-Barr virus (EBV) infection has been associated with some cases of IVL, although rarely[13] and only 1 report has examined the possible association of HHV-8 with IVL.[14] In this report, we describe the analyses of 5 cases of IVL for HHV-8 protein expression. One subject was human immunodeficiency virus-1 (HIV-1) seropositive and had KS, whereas the other 4 were HIV-1 seronegative and KS free.

Case reports

All cases were seen and treated at the University of Pittsburgh Medical Center. The use of all human specimens was approved by the University of Pittsburgh Institutional Review Board.

Case 1

A 45-year-old Hispanic homosexual man was diagnosed with HIV in 1989 and had a history of hepatitis B virus hepatomegaly, splenomegaly, and *Helicobacter pylori* infection. He was admitted to the hospital in 1994 for profound anemia and gastrointestinal (GI) consultation to elucidate the cause of GI bleeding. An esophagogastroduodenoscopy revealed thickened gastric folds with a possible lymphoma versus splenic vein thrombosis. A chronic *H pylori* infection was revealed by biopsy. The patient died 5 days following admission. Postmortem diagnosis consisted of AIDS, intravascular lymphomatosis, gastrointestinal hemorrhage, bilateral pneumonia, cerebral microabscess, anorexia, chronic anemia, and a history of opportunistic infections. The patient had KS on his feet, legs, thighs, trunk, and neck at autopsy. Intravascular lymphomatosis was discovered in the liver, small and large bowel, anus, and bilateral lower lungs at autopsy. Hepatomegaly, splenomegaly, and left apical lung bullae were discovered at autopsy as well.

Case 2

A 67-year-old man, race unknown, presented with a fever of unknown origin, nonspecific neurological symptoms without cutaneous lesions, or lymphadenopathy. Intravascular B-cell lymphoma was demonstrated pathologically in the peripheral blood, bone marrow, skeletal muscle, and sural nerve. At the time of biopsy, he was HIV negative.

Cases 3 to 5

There was limited clinical information for cases 3 to 5. All 3 were HIV negative at the time of biopsy. Case 3 was a 74-year-old man, race unknown, who was alive at the time of biopsy. A brain biopsy confirmed a B-cell IVL. Case 4 was a 76-year-old white woman who died of B-cell IVL. Confirmation of IVL was determined from an autopsy sample of spinal cord. Case 5 was a 68-year-old man, race unknown, who died of B-cell IVL. Confirmation of IVL was determined from an autopsy sample of the adrenal gland.

Methods

Immunohistochemistry

Paraffin sections were cut at 5 µm and mounted on Surgipath snowcoat X-tra microslides. The serial sections were deparaffinized in 3 xylene washes and rehydrated in a series of ethanol grades (100%, 95%, and 70%) to water. Antigen retrieval was performed using Target retrieval buffer (Dako, Carpinteria, CA) in a rice steamer for 40 (latency-associated nuclear antigen 1 [LANA-1]) or 20 (CD20, CD45) minutes. Blocking of endogenous peroxidases was done in methanol and H_2O_2 for 20 minutes. Nonspecific proteins were blocked with either normal rabbit serum (LANA-1) or normal goat serum (CD20, CD45) for 20 minutes at room temperature. Anti–ORF 73 (anti–LANA-1) rat monoclonal (ABI, Gaithersburg, MD), anti-CD20 mouse monoclonal, and anti-CD45 mouse monoclonal (Santa Cruz Biotechnology, Dallas, TX) were used as primary antibodies at a concentration of 1:1000 (LANA-1) and 1:500 (CD20, CD45). Incubation of primary antibodies occurred overnight at 4°C in a humidified chamber. Biotinylated rabbit anti-rat IgG and goat anti-mouse IgG were used as secondary antibodies (Vector labs, Burlingame, CA). Incubation of the secondary antibodies occurred for 60 minutes at room temperature in a humidified chamber. Vector ABC Elite and Vector NovaRED substrate (Vector Labs) were used to develop the staining for immunohistochemistry (IHC). Hematoxylin was used as the counterstain for IHC. In situ hybridization for Epstein-Barr virus–encoded RNAs (EBERs) was performed by the Presbyterian University Hospital Histology laboratory.

Results

HHV-8 immunohistochemistry

Sections from each IVL sample were stained with antibodies directed against the HHV-8 protein LANA-1. LANA-1 is the major viral protein expressed during latency,[1,15] and expression indicates the presence of a latent HHV-8 infection. To validate the IHC staining, LANA-1 staining was performed on KS lesions and a 1:1 mixture of body cavity–based lymphoma 1 (BCBL-1) cells and BJAB cells. BCBL-1 is a B-cell line that is latently infected with HHV-8[16] and BJAB is a well-studied HHV-8–negative B-cell line. As shown in Figure 1, LANA-1 staining in both the BCBL-1/BJAB mixture (Figure 1B) and a KS lesion (Figure 1D) demonstrates punctate, nuclear staining in agreement with other reports.[17,18]

Case 1

Lung. Immunohistochemical staining with LANA-1 antibody shows diffuse punctate nuclear staining in a large infiltrate in the lung (Figure 2B). The lack of significant cytoplasm and morphology of the LANA-1–positive nuclei suggest that the cells are mononuclear. The ×100 inset shows a multinucleated cell, with both nuclei positive for LANA-1 staining (Figure 2B). The cells were also CD20 and CD45 positive, indicating that they are B cells of a non-Hodgkin phenotype (Figure 2C, D). The Epstein-Barr virus early RNA (EBER) stain shows the presence of EBV infection in these cells (Figure 2E). The number of EBV-infected cells appears to be lower than the number of cells staining positive for LANA-1 (cf. Figure 2B to E).

Liver. The hematoxylin-eosin staining of the liver (Figure 3A) shows that the lymphoma cells are restricted to the sinusoidal cavities, straddling the interface area between the portal tract and hepatic lobule. No involvement of the adjacent tissue is seen. The LANA-1 staining (Figure 3B) shows punctuate nuclear staining of the nuclei with a decreased amount of cytoplasm in these positive cells. The LANA-1 positively stained cells in a similar fashion to the lymphoma cells, are within the sinusoidal cavities, and are not in the adjacent tissue.

Cases 2-5

All tissue sections from the HIV-negative IVL cases (cases 2-5) stained negative for LANA-1. Figure 4 shows representative staining of 2 of the non-HIV–infected IVL cases. Tissue sections from these cases (cases 2-5) were also negative for EBER staining, indicating that the IVL cells were not infected with EBV (data not shown).

Discussion and Conclusions

Human herpesvirus 8 is the causative agent of primary effusion lymphoma and KS in both AIDS and non-AIDS patients. In this study, we were interested in the association between HHV-8 and IVL. Intravascular lymphomatosis is a high-grade B-cell lymphoma defined as a proliferation of neoplastic mononuclear cells found within the lumina of the blood vessels (SEER Cancer Statistics). The incidence of B-cell non-Hodgkin lymphoma is 19.9/10 000 for men and 13.8/100 000 women in the United States, with IVL incidences being rare among this class (SEER Cancer Statistics).

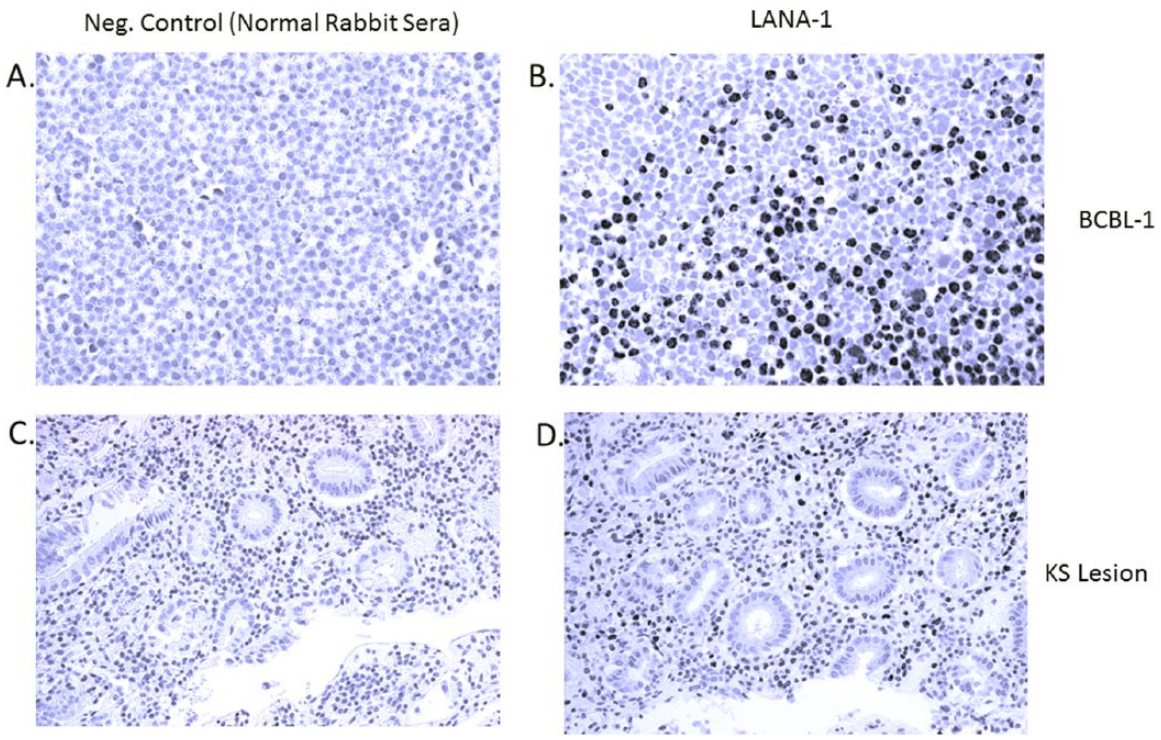

Figure 1. Validation of LANA-1 immunohistochemical staining. (A) BCBL-1/BJAB 1:1 mixture stained with normal rabbit sera, ×20; (B) BCBL-1/BJAB 1:1 mixture stained with LANA-1, ×20; (C) KS lesion stained with normal rabbit sera, ×20; D) KS lesion stained with LANA-1, ×20. BCBL-1 indicates of body cavity–based lymphoma 1; KS, Kaposi Sarcoma; LANA-1, latency-associated nuclear antigen 1.

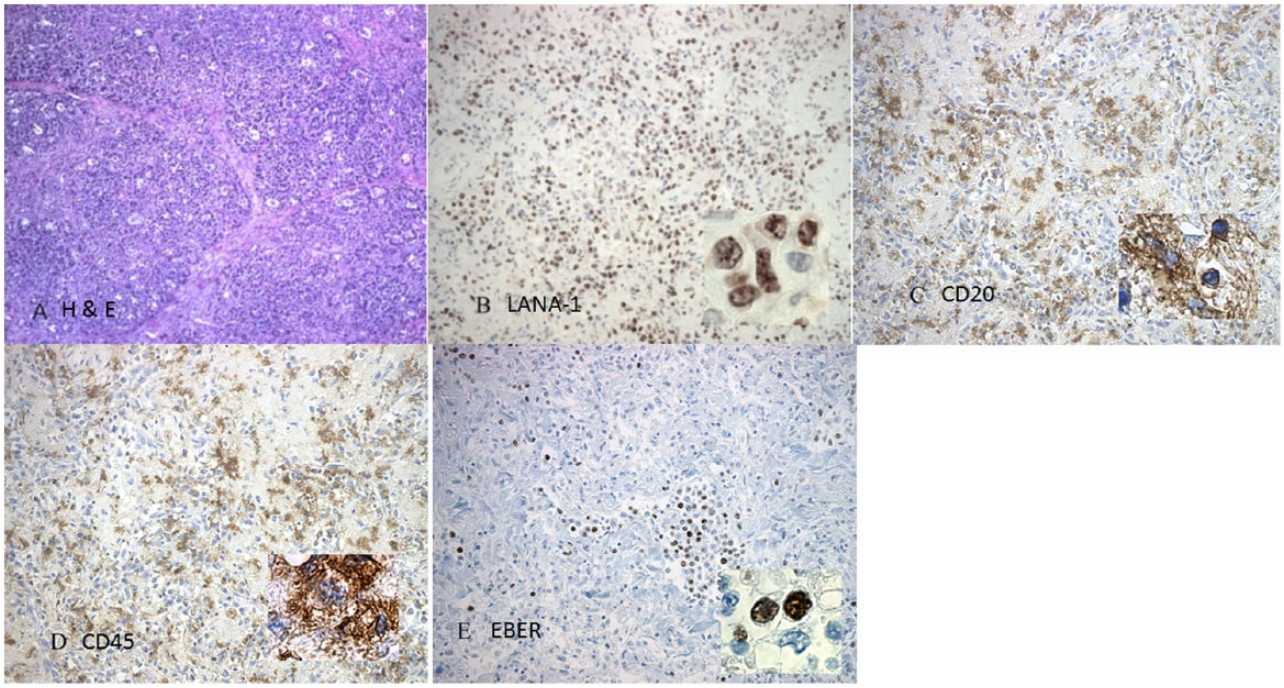

Figure 2. Immunohistochemical staining of the lung. (A) hematoxylin-eosin; (B) LANA-1 staining ×20, insert ×100; (C) CD20 staining ×20, insert ×100; (D) CD45 staining ×20, insert ×100; (E) EBER ISH ×20, insert ×100. EBER indicates Epstein-Barr virus early RNA; ISH, in situ hybridization; LANA-1, latency-associated nuclear antigen 1.

We tested 5 separate IVL cases for LANA-1 using IHC staining. Only case 1 was positive for HHV-8. Epstein-Barr virus is commonly found in NK cell–derived IVL but rarely in B-cell–derived IVL.[13] Human herpesvirus 8 is associated with other B-cell lymphomas, especially primary effusion lymphomas (PELs). Although most PELs are HHV-8 positive, some are also infected with EBV. To determine whether our HHV-8–positive IVL case was also EBV positive, we tested it for

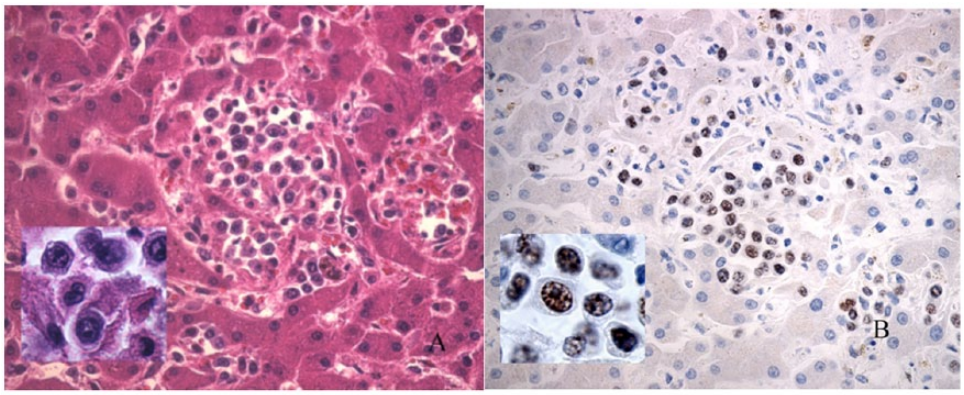

Figure 3. Immunohistochemical staining of the liver: (A) hematoxylin-eosin (H&E) ×40, insert ×100; (B) latency-associated nuclear antigen 1 (LANA-1) staining ×40, insert ×100.

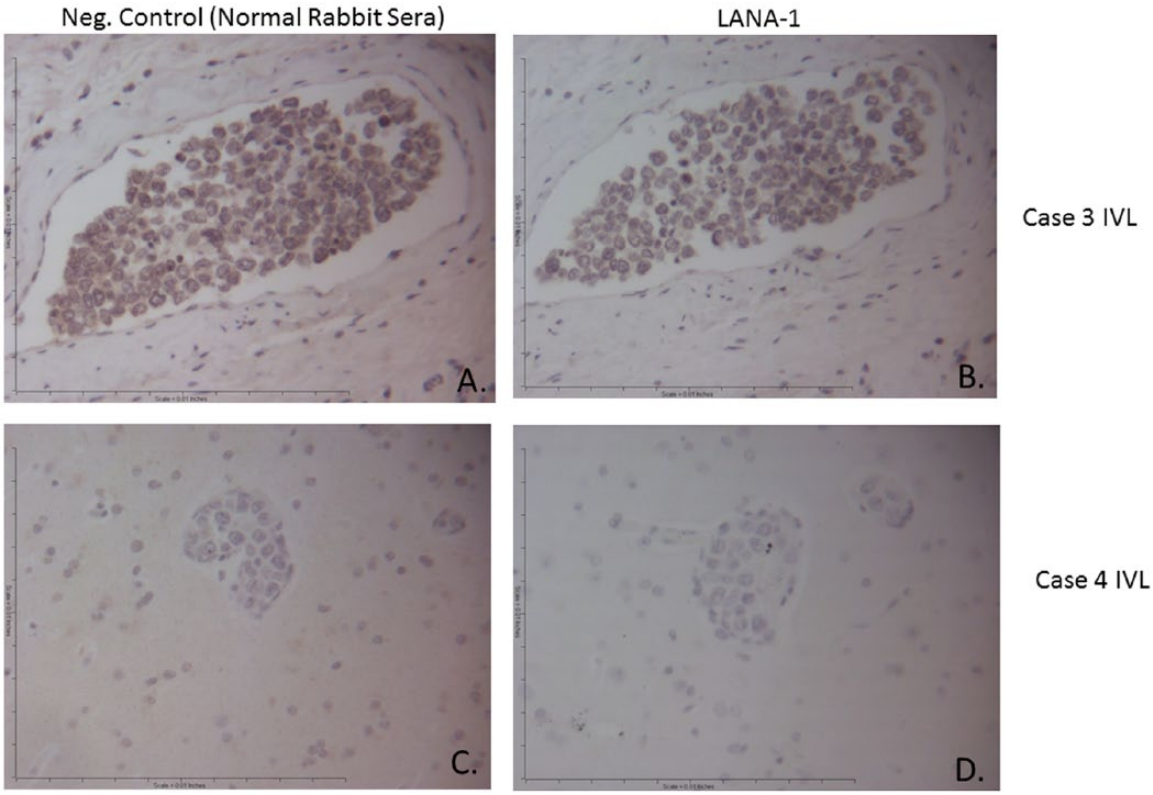

Figure 4. Immunohistochemical staining of IVL sections from non-HIV–infected cases. (A) IVL section stained with normal rabbit sera, ×40; (B) IVL section stained with LANA-1, ×40; (C) IVL section stained with normal rabbit sera, ×40; (D) IVL section stained with LANA-1, ×40. IVL indicates intravascular lymphomatosis; LANA-1, latency-associated nuclear antigen 1.

EBV via EBER staining and found it to be dually infected with both HHV-8 and EBV.

The LANA-1 staining of the lung and liver in case 1 shows the positive cells lacking large amounts of cytoplasm. This is suggestive of a cell of lymphoid origin. Also, the irregular shape of most of the positively stained cells is suggestive of lymphoid origin. The presence of CD20 and CD45 in such large quantities suggests that the lymphoma is of B-cell origin.

Our data support a previous report indicating the presence of HHV-8 in a single IVL case.[14] We have expanded these findings by demonstrating for the first time, using IHC, that HHV-8 is present in IVL cells as a latent infection (as measured by expression of LANA-1 in these cells). The HHV-8–positive IVL case reported in this study represents only the second case of an HHV-8 association. Both cases have been found in HIV-infected individuals. Further studies are required to determine whether HIV co-infection increases the risk of HHV-8–associated IVL and whether HHV-8 affects the pathogenicity and outcome of this unusual lymphoma.

Author Contributions

This work was carried out in collaboration with all authors. Developed the paper outline and wrote the first draft: FJJ, JDH. Provided samples, pathology reports and analyses of

immunohistochemistry staining: SMK, MAN. Performed immunohistochemistry: JDH, LDP. Analyzed data: FJJ, JDH. All authors contributed comments and editing of paper and reviewed and approved the final manuscript.

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