

Simultaneous occurrence of Epstein-Barr virus associated Hodgkin's disease and HHV-8 related multicentric Castleman's disease: a fortuitous event?

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Abstract

Previous serological or molecular studies by means of the polymerase chain reaction have failed to show an association between classic Hodgkin's disease (HD) and human herpesvirus 8 (HHV-8). Using immunohistochemistry, this study re-examines (with anti-LNA1 antibody) the possible association of HHV-8 with HD, particularly in human immunodeficiency virus (HIV) infected patients. HHV-8 was not detected in the Reed Sternberg cells of the cases examined (33 HIV negative and 17 HIV positive), thus confirming the lack of involvement of HHV-8 in HD. Interestingly, a case of HHV-8 positive multicentric Castleman's disease was associated with Epstein-Barr virus positive HD in the same lymph node, which was probably a fortuitous occurrence.

(*J Clin Pathol* 2001;54:790-791)

Keywords: human herpesvirus 8; Hodgkin's disease; multicentric Castleman's disease

Human herpesvirus 8 (HHV-8), also referred to as Kaposi's sarcoma herpesvirus, is a human herpesvirus recently identified by representational difference analysis that isolated genomic differences between Kaposi's sarcoma (KS) cells and normal skin tissue.¹ HHV-8 sequences have been detected by Southern blot or the polymerase chain (PCR) reaction in more than 90% of patients with KS, both those with and those without human immunodeficiency virus (HIV) infection (see Dupin *et al* and references therein²). HHV-8 sequences have also been detected in AIDS related, body cavity based lymphomas (also designated as primary effusion lymphoma) and multicentric Castleman's disease (see Dupin *et al* and references therein²). Despite some cases of post transplant lymphomas or plasmablastic lymphomas associated with multicentric Castleman's disease,³ it has not been possible to show a clear association between HHV-8 and other lymphoid tumours.

In immunocompetent patients, classic Hodgkin's disease (HD) is associated with Epstein-Barr virus (EBV) in approximately 50% of cases,⁴ whereas the incidence of EBV infection is close to 100% in HIV positive patients with HD.⁴ Previous serological⁵ or molecular studies (using PCR)^{6,7} have failed to show an association between HHV-8 and HD,

and to our knowledge, immunohistochemistry has not been used for this purpose.

Thus, using immunohistochemistry with a monoclonal antibody against LNA1^{2,3} (a latent nuclear antigen of HHV-8 encoded by viral open reading frame 73) we have re-examined the possibility that HHV-8 is associated with HD, at least in a small subset of cases. LNA1 appears to be a reliable marker for HHV-8 latent or lytic infection because it is constitutively expressed *in vitro* and *in vivo* and detectable by immunohistochemistry in all HHV-8 infected cells.^{2,3,8} We investigated 50 cases of classic HIV positive and HIV negative HD. We included a case of HHV-8 positive multicentric Castleman's disease associated with EBV positive HD in an HIV infected patient, which provides additional information to the case reports of an association between Castleman's and Hodgkin's disease (Abdel-Reheim *et al* and references therein⁹).

Material and methods

Lymph node biopsy specimens from 50 patients with classic HD were examined. The specimens were fixed in Duboscq Brasil liquid (acetic acid, 6.6%; 80% ethyl alcohol, 65.8%; 40% formol, 26.3%; picric acid, 0.4%; distilled water to 100%) or in 4% formol saline, and then routinely embedded in paraffin wax. The histopathological diagnosis of HD and HD subtype, and the EBV status of Reed Sternberg (RS) cells were based on immunomorphological criteria¹⁰ using anti-CD15 (clone C3D-1), anti-CD30 (clone Ber-H2), anti-epithelial membrane antigen (anti-EMA; clone E29), anti-latent membrane protein 1 (anti-LMP1; clone CS 1-4), anti-CD20 (clone L26), and anti-CD3 (clone T3) antibodies (Dako, Trappes, France).

The first series of lymph node biopsies came from 33 HIV negative patients: 17 cases of mixed cellularity HD (MCHD), 16 cases of nodular sclerosing HD (NSHD); age range, 21-82 years; male to female ratio, 14 : 19; 14 of these 33 cases were EBV positive.

The second series of lymph node biopsies came from 17 HIV infected patients: 15 cases of MCHD, two cases of HD difficult to subtype; age range, 10-45 years; male to female ratio, 16 : 1; 15 of these 17 cases were EBV associated. One of these patients presented with features of both HD and multicentric Castleman's disease in the same lymph node.

The presence of HHV-8 was investigated in all cases by standard immunohistochemical

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Accepted for publication
26 October 2000

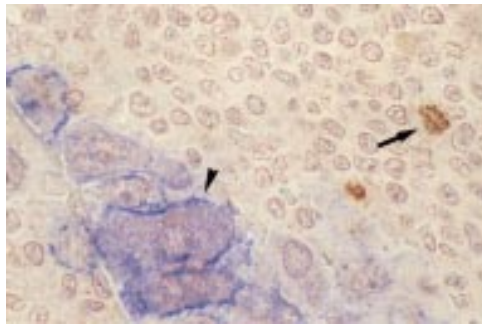


Figure 1 Lymph node from a human immunodeficiency virus positive patient presenting with multicentric Castleman's disease associated with Epstein-Barr virus (EBV) positive Hodgkin's disease (HD). Double staining showing the expression of latent nuclear antigen 1 (LNA1) and latent membrane protein 1 (LMP1). The periphery of the follicle contains a population of plasma cell-like lymphocytes (arrow) infected by human herpesvirus 8 (HHV-8) (LNA1+, LMP1-), some of which are close to the HD area (with many binucleated and multinucleated atypical cells and diagnostic Reed Sternberg cells) (arrowhead) infected by EBV (LMP1+, LNA1-). Anti-LNA1 antibody (clone LN53), peroxidase (brown); anti-LMP1 antibody (clone CS 1-4), alkaline phosphatase (blue); original magnification, $\times 1000$.

methods (avidin-biotin method) using a monoclonal antibody anti-LNA1 (clone LN53).^{2,3} The biopsy from the patient with features of HD and multicentric Castleman's disease in the same lymph node was double stained with anti-LNA1 (immunoperoxidase technique) followed by anti-LMP1 (alkaline phosphatase-anti-alkaline phosphatase procedure) (fig 1).

Results and discussion

We could not detect HHV-8 LNA1 protein in RS cells in the cases examined, thus confirming by immunohistochemistry the lack of involvement of HHV-8 in HD, irrespective of the patient's viral status. The lack of HHV-8 in EBV negative HD in HIV negative patients is well documented.⁶ The lack of association of EBV and HHV-8 in HIV positive patients indicates the involvement of different mechanisms of lymphomagenesis from that seen in AIDS related body cavity based lymphomas, the only known pathology involving both EBV and HHV-8 (see Dupin *et al* and references therein²). Furthermore, the lack of an association of HHV-8 in rare EBV negative HD seen in HIV positive patients (two cases in our study) reinforces the findings of our study.

To our knowledge, HHV-8 positive multicentric Castleman's disease associated with EBV-positive HD in an HIV infected patient has not been documented. Our study includes one such case; the patient was a 27 year old homosexual man with asymptomatic HIV infection diagnosed in 1992. He presented in 1995 with a six month history of fever. Physical examination revealed enlarged cervical lymph nodes; the spleen tip was palpable 4 cm below the costal margin. Computer tomography showed retroperitoneal lymphadenopathy. EBV positive HD (stage IIIB) and multicentric Castleman's disease were diagnosed on

examination of an enlarged cervical lymph node. Treatment with eight cycles of ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) and adjuvant irradiation could not control the progression of his HD and the patient died in 1997 from his lymphoma.

Morphologically, the cell populations infected with HHV-8 and with EBV were distinct and localised in different areas of the same lymph node. One area showed features of Castleman's disease with follicular hyperplasia associated with an "onion bulb" aspect of reticular dendritic cells and an interfollicular vascular proliferation. The periphery of the follicle contained a population of plasma cell-like elements infected by HHV-8 (LNA1+, LMP1-), with some of them being close to RS cells and variants (fig 1). Another area showed features of HD, with many binucleated and multinucleated atypical cells and diagnostic RS cells, all infected by EBV (CD15+/-, CD30+, CD20+/-, CD3-, LMP1+). No such lymphoma cell was infected by HHV-8 (LNA1-) (fig 1). This result appears to be specific because LNA1, which is detectable using immunohistochemistry with high sensitivity,^{2,3} is a specific marker of HHV-8 latent or lytic infection.⁸ Conversely, no HHV-8 infected cell was seen to be infected by EBV (fig 1). It is probable that, in our case, both multicentric Castleman's disease and HD are related to the underlying immunodeficiency. However, these results imply that the association of multicentric Castleman's disease and HD was merely fortuitous.

We thank Professor SM Chittal, University of St Johns, Newfoundland, Canada for critical reading of the manuscript and Dr E Buy, Hospital of Villeneuve sur Lot, France for providing patient data. This work was supported by grants from l'Institut Universitaire de France.

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