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Primary effusion lymphoma containing human herpesvirus 8 DNA in two AIDS patients with Kaposi's sarcoma

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ABSTRACT

Background and Objective. **Primary effusion lymphomas (PELs) containing Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8/HHV-8) DNA sequences represent a distinct but heterogeneous group of rare non-Hodgkin's lymphomas of null-cell phenotype/B-cell origin. We aimed to describe the clinicopathologic features of two human immunodeficiency virus (HIV)-related PELs occurring in homosexual men with Kaposi's sarcoma (KS).**

Design and Methods. **Thoracentesis was followed by morphologic plus immunophenotypic studies and molecular analysis of tumor cell DNA by means of combination of polymerase chain reaction and Southern blot analysis.**

Results. **Patients developed recurrent lymphomatous effusions lacking tissue involvement, in the context of severe immunodepression (CD4 count < 60/μL) and anti-retroviral therapy. The effusions disclosed an immunoblast-like population CD45/CD30$^+$, but B-cell- and T-cell-associated antigen negative, showing clonal immunoglobulin heavy chain gene rearrangements and harbouring HHV-8 DNA sequences. One case contained Epstein-Barr virus genome with no evidence of c-myc, bcl-2 and bcl-6 gene alterations. Both patients had aggressive disease.**

Interpretations and Conclusions. **These cases represent additional examples of PEL associated with HHV-8 and confirm that the group of HIV-positive homosexual men may be at highest risk for PEL.**

Key words: body-cavity-based-lymphoma, primary effusion lymphoma, Kaposi's sarcoma-associated herpesvirus, human herpesvirus-8, effusion, pleural cavity, Epstein-Barr virus

Body-cavity-based lymphomas (BCBLs) are unusual non-Hodgkin's lymphomas that grow mainly as lymphomatous effusions. Recent molecular studies[1,2] have identified two types of BCLBLs based on the presence or absence in tumor cells of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8/HHV-8) DNA sequences.

HHV-8-positive BCBLs have been designated primary effusion lymphomas (PELs).[3] They share common features (immunoblastic morphology, undetermined immunophenotype, clonal immunoglobulin gene rearrangement, lack of *c-myc*, *bcl-2*, and *bcl-6* rearrangements) and occur predominantly in human immunodeficiency virus (HIV)-positive homosexual males who are often coinfected with Epstein-Barr virus (EBV).[1-3] A previous history of Kaposi's sarcoma (KS) is infrequently reported in these patients.[1-10]

We describe two Italian men with HIV infection and disseminated KS who developed pleural and pleural-pericardial PEL containing HHV-8 DNA.

Materials and Methods

Patients

Case #1. A 42-year-old homosexual Italian male with a history of seropositivity for human immunodeficiency virus (HIV-1) since 1993 was hospitalized in December 1996 because of dyspnea, fever and fatigue. The patient had been treated with anti-retroviral therapy (zidovudine plus didanosine and the protease inhibitor Indinavir®). In November 1994 he developed a soft palate nodule that was surgically excised and diagnosed as KS, followed by cutaneous and gastric involvement. He received multiple cycles of α-interferon and chemotherapy (daunorubicin plus Vepesid®) but showed no clinical improvement. On admission, physical examination revealed disseminated cutaneous KS, hepatosplenomegaly and left medio-basal hypophonesis. Laboratory data disclosed: WBC 2.2×10^9/L with 45% neutrophils, 27% lymphocytes; hemoglobin 9.7 g/dL; 245×10^9/L platelets; lactic dehydrogenase (LDH) 438 U/L; albumin 2.2 g/dL. The CD4 count was 21/μL. Serum virological tests showed HBs-Ag, HBe-Ag and HCV-Ab negativity. A total body CT scan showed pericardial and bilateral pleural effusions, but no evidence of

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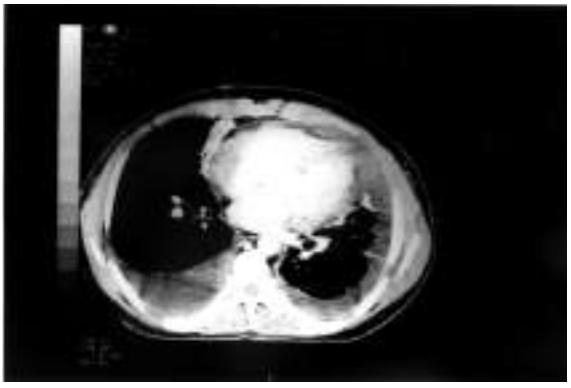

Figure 1. Thoracic CT scan showing massive bilateral pleural and pericardial effusions (case #1).

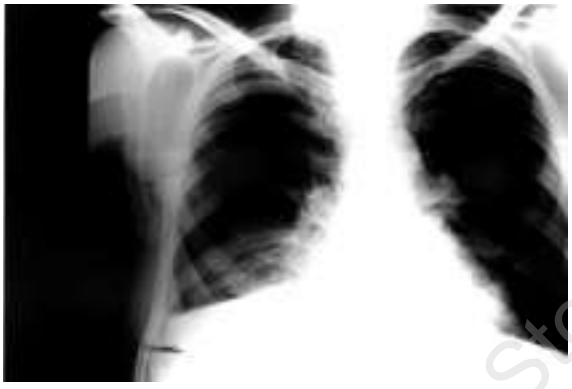

Figure 2. Chest X-ray showing right basal pleural effusion (case #2).

mass(es) (Figure 1). Cytological examination of pleural fluid showed a malignant effusion, probably lymphoma. The patient's clinical condition rapidly declined despite anti-HIV-1 therapy and periodic thoracenthesis, discouraging chemo- and radiotherapy. The patient died at home 2 months later.

Case #2. A 52-year-old HIV-1-positive homosexual Italian man was hospitalized in June 1997 because of dyspnea, fever (38°C), fatigue and anemia. In September 1996 he had developed purple cutaneous lesions of the left leg that were biopsied and diagnosed as KS; at the same time, HIV-1 seropositivity was discovered (CD4 cell count 59/μL). The patient was treated with combination anti-retroviral therapy (3TC plus D4T) and cotrimoxazole for *P.carinii* pneumonia prophylaxis, and multiple cycles of chemotherapy including vinblastine, adriamycin and bleomycin, without any clinical improvement. On admission in June 1997, physical examination revealed disseminated cutaneous KS of both legs accompanied by edema, hepatosplenomegaly and right basal hypophonesis. Laboratory studies disclosed: WBC 2.6×10^9/L with 46% neutrophils and 30% lymphocytes; hemoglobin 6.4 g/dL; 95×10^9/L platelets; albumin 2.3 g/dL. The CD4 count was 36/μL. Routine serologic tests excluded HBV and HCV infection. Chest X-ray showed mild unilateral pleural effusion (Figure 2). Thoracentesis disclosed malignant lymphoid cells. The clinical outcome was poor. He developed suppurative-necrotizing lesions on one leg and severe anemia despite blood transfusions. He died at home one month after drainage of the pleural fluid, in July 1997.

Morphologic and immunophenotypic analyses

Mononuclear cells derived from the pleural effusions were obtained after centrifugation on a Ficoll-Hypaque density gradient (1.077 g/L). Cytospin preparations were routinely stained with May-Grünwald-Giemsa and Papanicolaou stains. The immunophenotypic profile of the cell populations was determined by immunoperoxidase staining using a labelled straptavidin biotin (LSAB) method and the following monoclonal antibodies to CD19, CD20/L26, CD22, CD5, CD45, epithelial membrane antigen/EMA, CD30, HLA-DR, CD34, CD68, CD15/LeuM1, cytokeratin/CKMNF116. All reagents were supplied by Dakopatts (Glostrup, Denmark) except CD22, anti-LeuM1 (Becton-Dickinson, CA, USA).

Molecular studies

DNA analyses of tumor cells included:

1) Southern blot analysis of the immunoglobulin heavy chain (IgH), *c-myc*, *bcl-2* and *bcl-6* gene configurations in case #1;
2) polymerase chain reaction (PCR) amplification of HHV-8 DNA sequences in both cases using primers and PCR conditions described elsewhere,[1] and amplification of the IgH gene configuration in case #2;
3) PCR amplification of EBV DNA sequences in both cases by means of two different sets of specific primers. The first, encompassing the dyad symmetry element, was: 5'-ACGAAGGAGAATGAAGAAGCA GGCGAAGAT-3' (corresponding to nucleotides 8840-8869) and 5'-AGGGGTTCT CTGACTGTAGTTGACATCCTT-3' (corresponding to nucleotides 9427-9398); the second set, which spanned the internal region of EBNA2, has been reported elsewhere.[11] These sets of primers lack significant homologies with the HHV-8 sequences in the GenBank data base (accession number U75698, U75699, U757007). The specificity of the PCR products was confirmed by Southern blot hybridization.

Results

Morphology and immunophenotype

Both specimens demonstrated a polymorphic population of medium- to large-sized tumor cells intermixed with reactive cells that included small lymphocytes, macrophages, and rare mesothelial cells. Case #2 also showed a discrete number of polymorphonuclear leukocytes (eosinophils and neutrophils). Neoplastic cells were lying singly and were characterized by peripherally located, convoluted nuclei and abundant basophilic cytoplasm (Figure 3). Nucleoli were large, irregular in shape, and often multiple. Very large cells with pleomorphic nuclei and a clear Golgi zone, and bi- and multinucleated cells were also evident. Mitotic figures were fairly common. Nuclear fragmentation and karyopyknosis were additional findings. The immunophenotypic profile of tumor cells from both cases was as follows: $CD45^+$, $CD20^-$, $CD19^-$, $CD22^-$, $CD5^-$, EMA^+ (focal), $CD30^+$ (focal), $HLA\text{-}DR^+$, $cytokeratin^-$, $CD68^-$, $CD15^-$, $CD34^-$. The accompanying population of small lymphocytes was $CD5^+$ (T-cells); medium-sized cells were $cytokeratin^+$ (mesothelial cells) or $CD68^+$ (macrophages).

Molecular findings

Clonal rearrangement of the IgH gene was detected in both cases. Due to the low amount of available tumor cell DNA, case #2 could only be analyzed by PCR. HHV-8 DNA sequences were detected in DNA extracts from lymphoma cells from both patients (Figure 4). No variation from the germline configuration of control DNA was found by analyzing *c-myc*, *bcl-2* and *bcl-6* genes in case #1. The EBV genome was present in only one PEL (case #1).

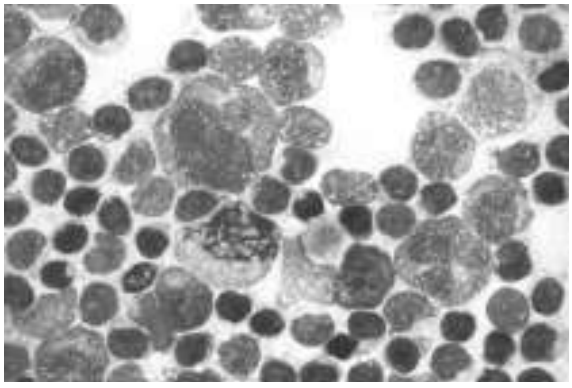

Figure 3. Pleural fluid sediment (May-Grünwald-Giemsa stain, ×1.400; case #1). Neoplastic effusion characterized by cellular heterogeneity, including large/medium lymphoid cells with convoluted, peripherally located nuclei and abundant basophilic cytoplasm. Small lymphocytes are also present. Note the abnormal mitotic figure.

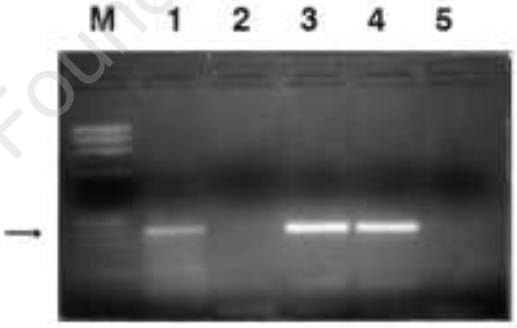

Figure 4. Results of PCR amplification of HHV-8 sequences. M, molecular weight marker IX (*ΦX174 Hae III digest*). Lane 1, DNA extracted from PEL cells from patient #2. Lane 2, DNA obtained from tumor cells from a patient with lymphoblastic lymphoma. Lane 3, DNA extracted from PEL cells from patient #1. Lane 4, positive control DNA obtained from a case of KS. Lane 5, negative control (all reagents plus water instead of DNA). The 233 bp amplification band corresponding to the HHV-8 sequences is indicated by the arrow.

Discussion

HHV-8 is consistently detected in Kaposi's sarcoma lesions (European-classic KS, African-endemic KS, iatrogenic KS, AIDS-KS), multicentric Castelman's disease and PELs.[12-15] These data suggest a pathogenetic role for HHV-8 in the development of these diseases.[16] Regarding hematological neoplasia, the presence of HHV-8 DNA in tumor cells seems to be confined to PELs, except for sporadic HHV-8-positive case descriptions of non-BCBLs.[17-19] Recently, it has been reported that HHV-8 can also support tumor growth by infecting stromal cells without infecting the neoplastic clone in multiple myeloma.[20]

We report two cases of HHV-8-positive lymphomatous effusions confined to serosal cavities (pleural and pleural-pericardial cavities) in HIV-1-seropositive homosexual men whose medical histories were noteworthy for untreatable generalized KS and severe immunodeficiency. Thoracentesis yielded an initial diagnosis of hematopoietic malignancy of uncertain lineage (probable lymphoma), since tumor cells expressed only CD45 and activation-associated antigens but lacked B-cell, T-cell, monocyte and myeloid lineage-restricted antigens. However, molecular analysis of tumor DNA revealed clonal IgH rearrangement consistent with B-cell differentiation, the presence of HHV-8 and, in the case also analyzed for known oncogene lesions, the absence of *c-myc*, *bcl-2* and *bcl-6* rearrangements. One of the two PELs contained EBV. The effusions were not associated with tumor mass(es). These cases fulfilled the diagnostic criteria for PEL.[3]

Relatively few PELs have been identified and characterized to date. Most cases have been reported in

North American patients,[3,4,6-8,21] with some others coming from European countries[9] including Italy.[5,22] The presence of HHV-8 has been documented in HIV-positive-[3,5,7-10,22] and, less frequently, HIV-negative-[2,4,6] related PELs. The vast majority of cases also contain EBV sequences.[3]

PELs are not necessarily closely linked to the development of KS, yet approximately one-third of patients with PEL have preexisisting KS lesions.[1-10] A lymphomatous effusion can be the primary manifestation of HHV-8 infection in both HIV-seropositive[5,9] and HIV-seronegative individuals,[4] or a late complication of non-AIDS KS[6] and AIDS-KS,[3] as occurred in our patients, who died shortly after the development of PEL. It can also appear concomitantly with the development of KS lesions.[9] To our knowledge, our cases are the first Italian PEL patients with KS; none of the other cases reported so far had a history of KS.[5]

It should be emphasized that the clinical course of the disease was unresponsive to therapy in both our patients. Recently, a complete remission of KS lesions was described in an AIDS-KS patient treated with an HIV-1 protease inhibitor,[23] whereas another *in vitro* study on cell lines established from a PEL patient did not show a direct inhibitory effect of some HIV-1 protease inhibitors on HHV-8 growth.[24] This seems to be confirmed by the aggressive clinical course of KS in our patient #1, who also developed a PEL during treatment with an HIV-1 protease inhibitor.

The reasons underlying the tendency of PELs to arise and grow in the body cavities remain unknown. It has been suggested that HHV-8 as a lymphotropic virus may infect a subset of B-lymphocytes with a peculiar homing pattern for body cavities.[8] Alternatively, HHV-8 itself might be responsible for the neoplastic growth pattern.[3] However, the characteristic of the effusion phenotype is shared by other BCBLs, namely Burkitt-type lymphomas,[3,25] pyothorax-associated lymphomas[26] and large-cell lymphomas,[27] that are HHV-8-negative. So far, HHV-8 sequences have not been found in other tumors related to body cavities.[28]

Body cavities are lined with mesothelial cells which constitutively express interleukin-6 (IL-6),[29] a cytokine with lymphocyte-stimulating capacities that is considered a B-cell differentiating factor. A viral homolog to the human IL-6 gene has recently been identified in HHV-8 DNA.[30] In the pathogenesis of PELs, mesothelial cells might provide a favorable environment for lymphoproliferation, and could potentially support lymphoma growth by producing IL-6.

Contributions and Acknowledgments

VA was responsible for the design, coordination and writing of the study, morphologic diagnosis, and day-to-day contact with partecipants. CMM, VG and MCS followed the patients clinically and contributed to specimen collection and writing of the paper. AF carried out EBV studies. AP performed DNA extractions, Southern blot analysis and PCR amplifications. FLC was responsible for the conception of the study, molecular analysis interpretation, funding, and writing of the paper.

The criteria used for the order in which the authors' names appear are the following: VA and FLC were the principal responsible for the study. CM,VG and MCS, AF and AP contributed to the execution of the study. All authors revised critically the article and approved the final version.

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Disclosures

Conflict of interest: none.

Redundant publications: no overlapping with previous papers.

Manuscript processing

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