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Anti-CD20 Monoclonal Antibody Treatment of Human Herpesvirus 8-Associated, Body Cavity-Based Lymphoma with an Unusual Phenotype in a Human Immunodeficiency Virus-Negative Patient

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Human herpesvirus 8 (HHV-8), or Kaposi's sarcoma-associated herpesvirus, is a gammaherpesvirus first detected in Kaposi's sarcoma tumor cells and subsequently in primary effusion lymphoma (PEL) tumor cells and peripheral blood mononuclear cells from PEL patients. PEL has been recognized as an individual nosologic entity based on its distinctive features and consistent association with HHV-8 infection. PEL is an unusual form of body cavity-based B-cell lymphoma (BCBL). It occurs predominantly in human immunodeficiency virus (HIV)-positive patients but occasionally also in elderly HIV-negative patients. We describe a case of PEL, with ascites, bilateral pleural effusions, and a small axillary lymphadenopathy, in a 72-year-old HIV-negative man. PCR performed on a lymph node specimen and in liquid effusion was positive for HHV-8 and negative for Epstein-Barr virus. The immunophenotype of the neoplastic cells was B $CD19^+$ $CD20^+$ $CD22^+$ with coexpression of CD10 and CD23 and with clonal kappa light chain rearrangement. The patient was treated with Rituximab, a chimeric (human-mouse) anti-CD20 monoclonal antibody. Thirteen months later, the patient continued in clinical remission. This is the first report of an HHV-8-associated BCBL in an HIV-negative patient in Argentina.

In 1994 Chang et al. (10) identified a new herpesvirus sequence in human immunodeficiency virus (HIV)-positive Kaposi's sarcoma dermopathy patients, named Kaposi's sarcoma-associated herpesvirus, or human herpesvirus type 8 (HHV-8). Later reports associated HHV-8 with a nonmalignant disease, Castleman's disease (19, 36), and with body cavity-based lymphoma (BCBL), also called primary effusion lymphoma (PEL) (7, 18, 31, 33). Since 1989 (15) most malignant effusion lymphomas reported have occurred in HIV-positive males (7, 31). PEL is a B-cell neoplasm characterized by infection of the tumor clone with HHV-8 and by liquid-filled body spaces without significant adenopathy. Although other lymphomas may develop cavity effusions, PEL is the only HHV-8-associated body cavity effusion lymphoma (11, 37). Recently, several PEL cases have been reported for HIV-negative individuals (5, 6, 9, 32, 34). PEL cells are usually coinfected with HHV-8 and Epstein-Barr virus (EBV) (7, 8, 31). However, there are cases of PEL cells infected with HHV-8 only (6, 9, 33). Because PEL is a malignant lymphoma, the treatment used for the past 15 years has been the standard treatment for non-Hodgkin lymphoma (NHL): cyclophosphamide, hydroxydoxorubicin, oncovin or vincristine, and prednisone (CHOP) in cyclic administration (22). If relapse or resistance to CHOP treatment occurs in cases of NHL, monoclonal-antibody therapy may be used (12). Satisfactory remissions of low-grade NHL have been obtained with monoclonal-antibody therapy (12, 13). There is no standard polychemotherapy for BCBL or PEL because of its very low incidence. Rituximab is a chimeric (human-mouse) monoclonal antibody that binds to the transmembrane antigen of the $CD20^+$ B cell, inducing apoptosis and complement-mediated cytotoxicity (17). In this work we report, for the first time in Argentina, a rare case of an HHV-8-associated BCBL with a B-cell phenotype in an HIV-negative male, in clinical remission after anti-CD20 treatment.

CASE REPORT

A 72-year-old man was referred to the Hematology Service at the Santojanni Hospital for investigation of pericardial and bilateral pleural effusions, plus ascites and chronic itching. Two years earlier he had presented with a lymphoproliferative disease, and biopsy of a 13-mm-diameter lymph node specimen showed a B $CD19^+$ $CD20^+$ $CD22^+$ immunophenotype with coexpression of CD10 and CD23 and with clonal kappa light chain rearrangement. After eight cycles of CHOP chemotherapy he was in clinical remission for 16 months, but prurigo remained. On examination, the patient was mildly dyspneic, with ascites and massive bilateral effusions, requiring several drainages. Lesions from scratching could be seen, but neither hepatosplenomegaly nor significant adenopathy was present. Laboratory tests showed eosinophilia (16%), a hemoglobin level of 115 g/liter, a white blood cell count of 5.7×10^9/liter,

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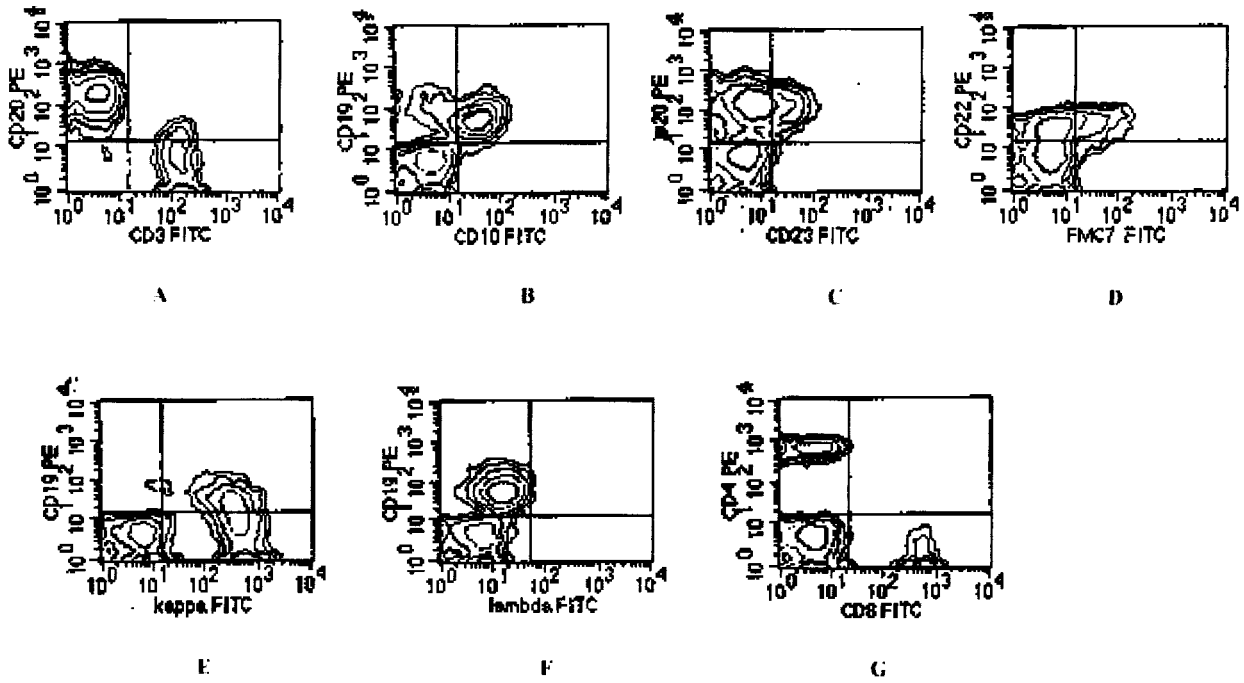

FIG. 1. Contour plots showing flow cytometry profile. (A) $CD20^+$ B-cell population (21%) and $CD3^+$ T-cell population (34%); (B) coexpression of CD19 and CD10; (C) coexpression of CD20 and CD23; (D) CD22 and FMC7 expression; (E) kappa light chain restriction; (F) normal expression of lambda chain; (G) T-cell population (21% CD4–12% CD8). FITC, fluorescein isothiocyanate; PE, phycoerythrin.

and a platelet count of 350×10^9/liter. Levels of markers for lymphoma evolution were increased as follows: lactic dehydrogenase, 740 IU (from 460); β2-microglobulin, 55 g/liter (from a range of 11 to 30). Results of additional studies, including serum protein electrophoresis and routine serum biochemistry (glucose, urea, albumin, cholesterol, glutamic-pyruvic transaminase, glutamic-oxalacetic transaminase, alkaline phosphatase, and creatinine), were normal. Results of enzyme-linked immunosorbent assay serology for HIV, HTLV 1 and 2, hepatitis B virus surface antigen, and hepatitis C virus were negative.

A chest computed-tomography scan showed bilateral pleural effusions; a computed-tomography scan of the abdomen revealed ascites with no hepatosplenomegaly and retroperitoneal adenopathies with diameters of less than 1.5 mm. A new lymph axillary node biopsy specimen was studied, and cytopathology was found, as was the case 2 years earlier. The immunophenotypic profile was 61% B lymphocytes and 34% T lymphocytes (Fig. 1A); the B-cell population expressed CD19 (Fig. 1B), CD20 (Fig 1C), and CD22 (Fig 1D), with coexpression of CD10 and CD23 antigens (Fig. 1B and C) and kappa light chain restriction (Fig. 1E). The T-cell population consisted of 21% $CD4^+$ cells and 12% $CD8^+$ cells (Fig. 1G). Bone marrow was not infiltrated by lymphoma cells. PCR was positive for HHV-8 (Fig. 2) and negative for EBV in both a lymph node biopsy specimen and liquid effusion. The patient underwent a four-cycle, 1-month Rituximab anti-CD20 treatment at the recommended dosage (26, 29). One month after the end of treatment, all effusions disappeared; itching and eosinophilia were also resolved. Seven months later, while in clinical remission, the patient was serologically tested by indirect immunofluorescence assay for HHV-8 (serum titer, 1/10) and EBV (serum titer, 1/40); nested PCR of peripheral blood mononuclear cells was positive for both HHV-8 and EBV. Thirteen months later, the patient continued in clinical remission.

MATERIALS AND METHODS

Cytological studies were performed on formalin fixed, paraffin-embedded lymph node specimens for hematoxylin-eosin staining. The immunophenotypic profile was determined by FACScan flow cytometry (Becton Dickinson Immunocytometry Systems) with fluorescein isothiocyanate- and phycoerythrin-conjugated monoclonal antibodies (Becton Dickinson) on the specimen obtained by aspiration biopsy.

PCR for HHV-8 was carried out on DNA extracted from 250 μl of fresh pleural effusion and from a paraffin-embedded lymph node specimen. PCR amplified the 233 bp of the KS 330 *Bam* region described by Chang et al. (10). PCR for EBV amplified the repeat segment in the *Bam*H-IW region, as previ-

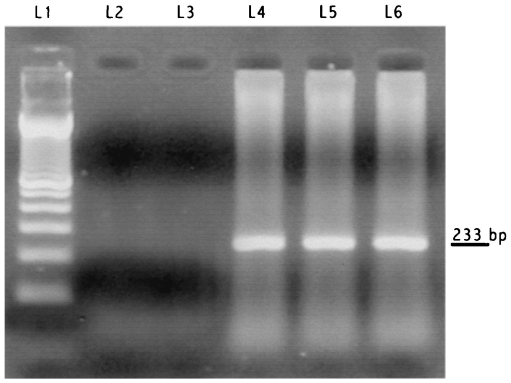

FIG. 2. PCR for HHV-8 (233 bp). Lanes: L1, molecular weight marker; L2, negative control (Raji cells positive for EBV and negative for HHV-8); L3, H_2O; L4, lymph node DNA; L5, pleural effusion DNA; L6, positive control (BCBL-1 cells [National Institutes of Health AIDS Research Program] positive for HHV-8 and negative for EBV).

ously described (35). Nested PCR for the same fragment of HHV-8 was carried out in peripheral lymphocytes as described elsewhere (25).

Ambulatory treatment with Rituximab was given at the recommended dose of 375 mg/m^2 of body surface in weekly infusions for 4 weeks (26, 27, 29) Sixty minutes before the treatment, a vial of acetaminophen was administered. Vital signs were monitored. The initial infusion of 50 mg/h was raised to 100 mg/h for 6 h in the 1st week. Subsequent administrations were reduced to 5 h.

Neither intolerance nor side effects were recorded.

RESULTS AND DISCUSSION

PEL was first described in 1989 by Knowles et al. (24), who observed major lymphomatous effusions in body cavities, usually growing in the absence of an identifiable tumor mass. Other investigators subsequently confirmed this observation (23, 38). In 1995, Cesarman et al. (7) reported the presence of HHV-8 in AIDS-related BCBL. Then the term PEL was suggested for AIDS-related and non-AIDS-related BCBLs that are characterized by effusions in body cavities without lymphadenopathy, tumor masses, or bone marrow involvement and are typically associated with HHV-8 (2, 3, 6, 9, 31). The main manifestations of lymphoma in the case reported in this paper were pleural effusions and ascites. The volume and locations of these effusions prompted us to review the case and to look for HHV-8 in effusions and lymph node specimens. Reports describing early lymph node involvement of HHV-8 DNA in an HIV-negative PEL patient (1), small intra-abdominal solid tumors detected by imaging in a few cases (30), and HHV-8 DNA in fluid effusions (7, 21, 31) support the possibility that HHV-8 may play a role in PEL pathogenesis. Some cases of PEL in HIV-negative patients have been reported previously (1, 5, 9, 14, 21, 32). The average age of these patients was 72 years, coincident with the age of our patient. Although other investigators have reported that the main features of PEL are large, anaplastic CD19$^-$ CD20$^-$ cells (9, 16, 23, 31, 38), there are CD20$^+$ (33) and CD19/20$^+$ PELs (6, 9, 20), and some cases with an intermediate lymphocytic cytomorphology have been described (9). Moreover, on the basis of immunoglobulin VH gene mutational analysis, it has been suggested that PEL may not be restricted to one stage of B-cell differentiation but may represent transformation of B cells at different stages of ontogeny (28). In an animal model, mice injected with lymphoma cells from a PEL patient developed small intra-abdominal tumors (4). It has also been proposed that the clinical spectrum of manifestations at presentation of PEL may be wider than initially described (1). On the basis of the reports cited and the clinical and molecular results obtained, the diagnosis for this patient was PEL. Because of its very low incidence, there is no standard polychemotherapy for BCBL or PEL. Most PEL patients do not respond to CHOP therapy (1). Recently, a PEL with a B-cell phenotype successfully treated with prednisone was reported (20), but the patient died 15 months after PEL diagnosis. There are reports of satisfactory remissions in low-grade NHL (12, 13) and in bone marrow transplant purging following treatment with anti-B-cell monoclonal antibodies. Rituximab is a chimeric (human-mouse) monoclonal antibody that binds to the CD20 B cell's transmembrane antigen, inducing apoptosis and complement-dependent cytotoxicity (17). We chose Rituximab therapy for this case based on previous CHOP treatment failure and the CD20$^+$ immunophenotype.

We report here a case of a patient with an HHV-8-associated BCBL, with a B-cell phenotype, treated with an anti-CD20 monoclonal antibody. In view of the poor results of present schemes, monoclonal therapy may be another modality of treatment for PEL patients.

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