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 CD25 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). Since 1989 (15) most malignant effusion lymphomas have reported 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, hydroxydaunorubicin, 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 × 10⁹/liter,
and a platelet count of 350 × 10⁹/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 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 BamH-IW region, as previ-
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, Cesarsan 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 in effusions and lymph node specimens. Reports described 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 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 cytology 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 oncogeny (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.

REFERENCES

1. Arias, S., D. Benharroch, L. Lupu, B. Davidovici, N. Dupin, and C. Bosshoff. 2000. Early peripheral lymph node involvement of human herpesvirus 8-associated body cavity-based lymphoma in a human immunodeficiency virus-positive patient. Arch. Pathol. Lab. Med. 124:753–755.
2. Ascoli, V., C. C. Scalzo, C. Danese, K. Vacca, A. Pistilli, and F. Lo Coco. 1999. Human herpesvirus 8-associated primary effusion lymphoma of the pleural cavity in HIV-negative elderly men. Eur. Respir. J. 14:1231–1234.
3. Assou, H., J. Said, R. Yang, R. Munkner, D. J. Park, N. Kamada, and H. P. Koedler. 1998. Mechanisms of growth control of Kaposi’s sarcoma-associated herpesvirus-associated primary effusion lymphoma cells. Blood 91: 2475–2481.
4. Bosshoff, C., S. J. Gao, L. E. Healy, S. Matthews, A. J. Thomas, L. Coignet, R. A. Warnke, J. A. Strauchen, E. Matutes, O. W. Kamel, P. S. Moore, R. A. Weiss, and Y. Chang. 1998. Establishing a KSHV+ cell line (BCP-1) from peripheral blood and characterizing its growth in NOD/SCID mice. Blood 91: 1671–1679.
5. Bosshoff, C., D. Whithby, T. Hatzioannou, C. Fisher, J. van der Walt, A. Hatzakis, R. Weiss, and T. Schulz. 1995. Kaposi’s sarcoma-associated herpesvirus in HIV-negative Kaposi’s sarcoma. Lancet 345:1043–1044.
6. Cesarman, A., A. Gloghini, E. Vaccher, V. Zagonel, C. Pastore, P. Dalla Palma, F. Branz, G. Saglio, R. Volpe, U. Tirelli, and G. Gaidano. 1996. Kaposi’s sarcoma-associated herpesvirus DNA sequences in AIDS-related and AIDS-unrelated lymphomatous effusions. Br. J. Haematol. 94:533–543.
7. Cesarsan, E., Y. Chang, P. Moore, J. Said, and D. Knowles. 1995. Kaposi’s sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body cavity-based lymphomas. N. Engl. J. Med. 332:1186–1191.
8. Cesarsan, E., P. S. Moore, P. H. Rao, G. Inghirami, D. M. Knowles, and Y. Chang. 1995. In vitro establishment and characterization of two acquired immunodeficiency syndrome-related lymphoma cell lines (BC-1 and BC-2) containing Kaposi’s sarcoma-associated herpesvirus-like (KSHV) sequences. Blood 86:2708–2714.
9. Cesarsan, E., R. G. Nudor, K. Aozasa, G. Delsol, J. Said, and W. D. Knowles. 1996. Kaposi’s sarcoma-associated herpesvirus in non-AIDS-related lymphomas occurring in body cavities. Am. J. Pathol. 149:53–57.
10. Chang, Y., E. Cesarsan, M. S. Pessin, F. Lee, J. Culpepper, D. M. Knowles, and P. S. Moore. 1994. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi’s sarcoma. Science 266:1865–1869.
11. Cobo, F., E. Montserrat, and E. Campo. 1997. Linfonoma primario de cavidades: una nueva entidad clinicopatológica. Med. Clin. 109:712–714.
12. Cesarsan, E., A. J. Grillo-Lopez, and C. White. 1998. Rituximab/chemoimmunotherapy in patients with low-grade lymphoma: progression-free survival after 3 years median follow-up. Blood 94(Suppl. 1):99A.
13. Cushman, M. S., A. J. Grillo-Lopez, C. A. White, M. Saleh, L. Gordon, A. F. Leach, C. Jonas, D. Luskinstein, R. Dallaire, and C. Varrns. 1999. Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. J. Clin. Oncol. 17:268–276.
14. Dauca, G., R. Fischetti, T. Motta, R. Facchinetti, B. Chioldini, G. Borleri, G. Gavazzeni, T. Barbui, and A. Rambaldi. 1999. Primary effusion lymphoma after heart transplantation: a new entity associated with human herpesvirus 8. Leukemia 13:664–670.
15. Feiner, H. D., et al. 1989. Frequent null cell status of lymphomas in the pleura in HIV+ patients. Lab. Invest. 60:26A.
16. Green, L., C. Espiritu, M. Ladanui, R. Chaponda, R. Wielorek, L. Gallo, and H. Feiner. 1995. Primary lymphomatous effusions in AIDS: a morphological, immunophenotypic, and molecular study. Mod. Pathol. 8:535–45.
17. Harjupanpa, A., S. Junnikkala, and S. Meri. 2000. Rituximab (anti-CD20) therapy of B-cell lymphomas: direct complement killing is superior to cellular effector mechanisms. Scand. J. Immunol. 51:634–641.
18. Harris, N. L., E. S. Jaffe, H. Stein, P. M. Banks, J. K. Chan, M. L. Cleary, G. Delsol, C. De Wolf-Peeters, B. Falini, and K. C. Gatter. 1994. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 84:1361–1392.
19. Humphrey, R. W., D. A. Davis, F. M. Newcomb, and R. Yarchoan. 1998. Human herpesvirus 8 (HHV-8) in the pathogenesis of Kaposi’s sarcoma and other diseases. Leukemia Lymphoma 28:255–264.
20. Iwahashi, M., S. Iida, S. Sako, S. Inoue, H. Kikuchi, E. Otsuka, and M. Nasu. 2000. Primary effusion lymphoma with B-cell phenotype. Am. J. Hematol. 64:317–318.
21. Jones, D., M. E. Ballestas, K. M. Kaye, J. M. Gulizia, G. L. Winters, J. Fletcher, D. T. Scadden, and J. C. Aster. 1996. Primary-effusion lymphoma and Kaposi’s sarcoma in a cardiac-transplant recipient. N. Engl. J. Med. 339: 444–449.
22. Jones, S., E., et al. 1979. Superiority of adriamycin-containing combination...
chemotherapy in the treatment of diffuse lymphoma: a Southwest Oncology Group study. Cancer 43:417–425.

23. Karcher, D. S., F. Dawkins, C. T. Garret, and R. S. Schulof. 1992. Body cavity-based non-Hodgkin’s lymphoma (NHL) in HIV-infected patients: B-cell lymphoma with unusual clinical, immunophenotypic, and genotypic features. Lab. Investig. 66:30A.

24. Knowles, D., G. Inghirami, A. Ubriaco, and R. Dalla Favera. 1989. Molecular genetic analysis of three AIDS-associated neoplasms of uncertain lineage demonstrates their B-cell derivation and the possible pathogenetic role of the Epstein-Barr virus. Blood 73:792–799.

25. Lock, M., P. Griffiths, and V. Emery. 1997. Development of a quantitative competitive polymerase chain reaction for human herpesvirus 8. J. Virol. Methods 64:19–26.

26. Maloney, D. G., T. M. Liles, D. K. Czerwinski, C. Waldichuk, J. Rosenberg, A. Grillo-López, and R. Levy. 1994. Phase I clinical trial using escalating single-dose infusion of chimeric anti-CD20 monoclonal antibody (IDEC-C2B8) in patients with recurrent B-cell lymphoma. Blood 84:2457–2461.

27. Maloney, D., and O. Press. 1998. Newer treatments for non-Hodgkin’s lymphoma: monoclonal antibodies. Oncology 12(Suppl. 8):63–76.

28. Matolcsy, A., R. Nador, E. Cesarman, and D. Knowles. 1998. Immunoglobulin VH gene mutational analysis suggests that primary effusion lymphomas derive from different stages of B-cell maturation. Am. J. Pathol. 153:1609–1614.

29. McLaughlin, P., A. Grillo López, and M. Czuzman. 1998. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J. Clin. Oncol. 16:2825–2833.

30. Morassut, S., E. Vaccher, L. Balestrieri, A. Gloghini, G. Gaidano, R. Volpe, U. Tirelli, and A. Carbone. 1997. HIV-associated human herpesvirus 8-positive primary lymphomatous effusions: radiologic findings in six patients. Radiology 205:459–463.

31. Nador, R., E. Cesarman, A. Chadburn, D. B. Dawson, M. Q. Ansari, J. Said, and D. M. Knowles. 1996. Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi’s sarcoma-associated herpesvirus. Blood 88:643–656.

32. Nador, R., E. Cesarman, D. Knowles, and J. W. Said. 1995. Herpes-like DNA sequences in a body cavity-based lymphoma in an HIV-negative patient. N. Engl. J. Med. 333:943.

33. Said, J., K. Chien, S. Takeuchi, T. Tasaka, H. Asou, S. de Vos, E. Cesarman, D. Knowles, and H. P. Koehler. 1996. Kaposi’s sarcoma-associated herpesvirus (KSHV or HHV-8) in primary effusion lymphoma: ultrastructural demonstration of herpesvirus in lymphoma cells. Blood 87:4937–4943.

34. Said, J., T. Tasaki, S. Takeuchi, H. Asou, S. de Vos, E. Cesarman, D. M. Knowles, and H. P. Koehler. 1996. Primary effusion lymphoma in women: report of two cases of Kaposi’s sarcoma herpesvirus-associated effusion-based lymphoma in human immunodeficiency virus-negative women. Blood 88:3124–3128.

35. Saito, I., B. Servienius, T. Compton, and R. Fox. 1989. Detection of Epstein-Barr virus DNA by polymerase chain reaction in blood and tissue biopsies from patients with Sjogren’s syndrome. J. Exp. Med. 169:2191–2198.

36. Teruya-Feldstein, J., P. Zauber, J. Setsuda, E. Berman, L. Sorbara, M. Raffeld, G. Tosato, and E. Jaffe. 1998. Expression of human herpesvirus-8 oncogene and cytokine homologues in an HIV-seronegative patient with multicentric Castleman’s disease and primary effusion lymphoma. Lab. Investig. 78:1637–1642.

37. Uphoff, C., A. Carbone, G. Gaidano, and H. Drexler. 1998. HHV-8 infection is specific for cell lines derived from primary effusion (body cavity-based) lymphomas. Leukemia 12:1806–1809.

38. Walts, A. E., J. P. Shintaku, and J. W. Said. 1990. Diagnosis of malignant lymphoma in effusion from patients with AIDS by gene rearrangement. Am. J. Clin. Pathol. 94:170–175.