Brief report

EBV-positive immunodeficiency lymphoma after alemtuzumab-CHOP therapy for peripheral T-cell lymphoma

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Chemotherapy with alemtuzumab and the combination of cyclophosphamide, adriamycin, oncovin, and prednisone (CHOP) has become experimental trial therapy for aggressive T-cell lymphoma. Several multicenter phase 3 trials will incorporate this scheme. As part of an ongoing phase 2 trial in which we recently treated 20 patients with 8 cycles of CHOP every 2 weeks with 3 additional doses of 30 mg alemtuzumab per cycle, we observed the development of Epstein-Barr virus (EBV)-positive lymphoproliferative disease, after completion of the immunochemotherapy in 3 patients with peripheral T-cell lymphoma. Because the occurrence of EBV-positive lymphoproliferative disease is rare after alemtuzumab monotherapy, such as is given for chronic lymphocytic leukemia, we think that early reporting of this potential side effect is warranted. It may be caused by intrinsic T-cell defects in patients with T-cell lymphoma, or by the combination of alemtuzumab with CHOP chemotherapy. (Blood. 2008;112:1039-1041)

Introduction

The poor prognosis of peripheral T-cell lymphoma has generated new trials with immunochemotherapy consisting of the anti-CD52 monoclonal antibody alemtuzumab combined with CHOP (cyclophosphamide, adriamycin, oncovin, prednisone) chemotherapy.1 Although increased infection rates have been reported in these alemtuzumab-CHOP trials, toxicity thus far has been manageable.2 Based on these preliminary data, 2 alemtuzumab-based phase 3 trials (ACT1 and ACT2) for patients with peripheral T-cell lymphoma have recently been launched by a large European Intergroup initiative.

We encountered secondary Epstein-Barr virus (EBV)-related lymphoma in 3 patients shortly after completion of alemtuzumab-CHOP treatment. All patients participated in a phase 2 trial (Dutch-Belgian Cooperative Trial Group Hematology Oncology [HOVON] 69 study) in which 20 patients participated, consisting of 8 CHOP cycles given at 14-day intervals combined with 3 doses of 30 mg of alemtuzumab per cycle. The HOVON 69 phase 2 study was reviewed by the Central Dutch Medical Ethical Committee located at the University Medical Center Groningen and by the Central Clinical Trial Office. Both gave permission for conducting the trial. All patients gave informed consent in conformance with the Declaration of Helsinki.

Methods

Case reports

Case 1. In May 2006, a 41-year-old man presented with peripheral T-cell lymphoma, unspecified, stage IIIA, localized in retroperitoneal lymph nodes, spleen, and mediastinum. T-cell receptor (TCR) clonality analysis showed clonal rearrangements of TCR-β and TCR-γ genes. The lymphoma was EBV negative by in situ hybridization. Whole blood EBV-DNA copies were below detection level. He received 8 alemtuzumab-CHOP-14 cycles together with co-trimoxazol, valaciclovir, and fluconazol prophylaxis until October 2006. Treatment was complicated by neutropenic fever and recurrent cytomegalovirus (CMV) reactivation, treated with ganciclovir. In November 2006, CT scans showed a complete response. EBV-DNA viral load copies, which had remained below detection level until then, subsequently increased up to levels of 3 to 10 × 103 copies/mL (normal < 1 × 103). In December 2006, he developed ulcerative duodenitis. In January 2007 a CT scan showed a tumor mass obstructing the duodenal loop; EBV-DNA levels had increased (574 × 103). Rituximab was started, without improvement. Multiple biopsies from the duodenum and stomach in January and March 2007 revealed an EBV-encoded RNA (EBER)-positive T-cell lymphoproliferative disorder with massive intraepithelial accumulation of EBER-positive and CD2, CD3, and CD5 positive but CD4and CD8-negative T cells that were oligoclonal as analyzed by TCR-β and TCR-γ polymerase chain reaction (PCR). In April 2007, a biopsy was performed from the tumoral mass around the duodenum. This showed an EBV-negative T-cell lymphoma that on TCR-β and TCR-γ gene analysis was clonally different from the first T-cell lymphoma diagnosed in 2006. He was treated with salvage chemotherapy (dexamethason, cytarabin, cisplatin). However, he developed massive hemophagocytic syndrome and died in July 2007. Autopsy was not performed.

Case 2. In November 2005, a 32-year-old woman with a history of stable multiple sclerosis was diagnosed with a panniculitis-like T-cell lymphoma, TCR-α/β phenotype, stage IV with extensive localizations in the skin and lymph nodes on both sides of the diaphragm. EBV antibodies or viral EBV-DNA load were not measured. T-cell clonality analysis and in situ hybridization for EBV were not performed. She received 8 cycles of alemtuzumab-CHOP-14 until March 2006. Prophylaxis was given with co-trimoxazol, valacyclovir, and fluconazol during and after completion of therapy because of persistent severe T-cell lymphocytopenia. A PET-CT scan after completion of therapy showed a complete remission. In August 2006, she had an epileptic seizure. A large intracerebral mass was biopsied,
which yielded CD20+ necrotic tumor cells. EBV serology was strongly positive with IgG and IgM-antibodies for EBV–early antigen and IgG antibodies for EBV–nuclear antigen; whole blood EBV viral load showed less than 2000 copies/mL. EBER in situ hybridization was negative but possibly false-negative because of necrosis. She received radiotherapy, followed by intravenous rituximab maintenance. In November 2006, she developed a pharyngeal diffuse large B-cell lymphoma that was CD20-negative (directly after rituximab), and EBV strongly positive. B-cell clonality was not assessed. Whole blood EBV viral load was not measured. Because of poor performance, involved-field radiotherapy was administered, and she received palliative treatment with prednisone. In April 2007, prednisone was tapered because of gradual improvement. In August 2007, however, she developed subcutaneous lesions that showed both a relapse of the original panniculitis-like T-cell lymphoma and an EBV-positive B-cell plasmacytoid lymphoproliferative disease with clonal expression of cytoplasmic kappa light chains. PCR clonality analysis showed clonal rearrangement of TCR-β and –γ genes. She refused further treatment. As of January 2008, the patient is still alive and even improving. All subcutaneous lesions have disappeared spontaneously. Her CD4 and CD8 counts remain severely depressed.

**Case 3.** In January 2006, a 59-year-old man developed stage III angioimmunoblastic T-cell lymphoma (AILT) with involvement of multiple lymph nodes and the spleen. At that time, no atypical B-cell proliferation was seen and only scattered EBV-positive (presumably B) cells were detected in the lesional tissue. Clonality analysis was not performed. Whole-blood EBV-DNA load was negative. He received 6 alemtuzumab-CHOP-14 cycles together with co-trimoxazol, valaciclovir, and fluconazol prophylaxis until April 2006. Because of recurrent CMV reactivations, the last 2 cycles were not given. A PET and CT scan disclosed a complete remission. In April 2007, a CMV retinitis was treated by foscarinet, and EBV-DNA was still below detection level. In December 2007, he developed fever, dyspnea, generalized lymphadenopathy, splenomegaly, multiple skin nodules, pleural fluid, and ascites. A lymph node biopsy disclosed a relapse of the original AILT associated with an EBV-positive CD20-positive B-cell lymphoproliferative disease from which he died soon after. EBV-DNA load measured 991 × 10^3 copies/mL. The EBV-positive B-cell lymphoproliferative disease was mononclonal by PCR analysis.

**Pathology**

**EBV assessment.** The detection of EBER-1 and –2 was performed by in situ hybridization on paraffin-embedded tissue sections using a fluorescein-conjugated EBER peptide nucleic acid probe (DakoCytomation Denmark, Glostrup, Denmark). The reaction was visualized with alkaline phosphatase conjugated anti-fluorescein isothiocyanate sheep IgG Fab fragments (Roche Diagnostics, Mannheim, Germany) followed by 5-bromo-4-chloro-3-indolyl-phosphate, 4-nitrobluetetrazolium (Roche Diagnostics) and MgCl2 incubation. Clonality of the EBV episomes was not investigated.

**B-cell and T-cell clonality assessment.** Immunoglobulin and TCR clonality analysis was performed on DNA extracted from paraffin-embedded tissue by multiplex IgH and TCR-β and –γ PCR according to the BIOMED-2 protocol.3 The PCR products were detected by heteroduplex analysis or GeneScanning (TCR-β and –γ analysis courtesy of Prof J. J. van Dongen, Erasmus Medical Center, Rotterdam, The Netherlands).

**Discussion**

EBV-lymphoproliferative disease is a familiar complication after organ transplantation and is related to the severity of immunosuppression.4-5 Most cases are of B-cell type, but T-cell EBV-lymphoproliferative disease can also occur.8 A comparable lymphoproliferative disorder may occur in the context of autoimmune disorders treated with immunosuppressive or immunomodulating agents. Here we report the occurrence of EBV-lymphoproliferative disease after strong suppressive immunochemotherapy consisting of alemtuzumab-CHOP for peripheral T-cell lymphoma.

Alemtuzumab (Campath-1H) targets CD52, which is present on virtually all B and T lymphocytes, monocytes, and natural killer (NK) cells and is therefore highly immunosuppressive. Particularly, the prolonged T-cell deficiency can lead to opportunistic infections.7,8 Alemtuzumab has been widely used in patients with chronic lymphocytic leukemia,9 T-cell prolymphocytic leukemia,10-12 and more recently T-cell lymphoma.1,2,12-14

Despite the large numbers of patients with chronic lymphocytic leukemia or T-prolymphocytic leukemia treated, the occurrence of EBV-lymphoproliferative disease after therapy with alemtuzumab seems very rare.1,5,17 A patient with refractory Sézary syndrome, treated with alemtuzumab, who developed fatal adenoviral and enteroviral infections and EBV-positive large B-cell lymphoma was recently published.18

The fact that we observed several cases with EBV-lymphoproliferative disease after the completion of alemtuzumab-CHOP therapy for T-cell lymphoma is remarkable. Case 3 had AILT, which is more often accompanied by secondary EBV-positive B-cell transformations19,20; however, both other cases had non-AILT T-cell lymphoma. Our observation suggests a relationship between this complication, the alemtuzumab-CHOP regimen, and T-cell lymphoma.

A relationship between EBV-positive B-cell lymphoma and T-cell lymphoma has been described, not only in AILT, but also in peripheral T-cell lymphoma.21 Of 600 cases with nodal T-cell lymphoma, 17 cases with secondary EBV-associated B-cell lymphoma were reported: 13 with AILT, one peripheral T-cell lymphoma, and 3 cases with coexisting EBV-related B–non-Hodgkin lymphoma as part of a composite lymphoma. These published cases were all treated before alemtuzumab became part of the therapy. The HOVON 69 alemtuzumab-CHOP scheme contained a very intensive alemtuzumab dose (90 mg per CHOP-14 cycle) compared with other trials in T-cell lymphoma. Within the forthcoming ACT1 and ACT2 trials, alemtuzumab-CHOP-14, be it with less alemtuzumab (60 mg per CHOP-14 cycle), will be the experimental therapy for patients with peripheral T–non-Hodgkin lymphoma. It needs to be seen whether we will more often encounter EBV-driven B-cell and/or T-cell lymphomas if alemtuzumab-CHOP becomes standard therapy for patients with T-cell lymphoma. Although the poor outcome of T-cell lymphoma justifies high-risk therapy modalities, awareness of this complication is important. In addition, EBV-DNA monitoring could lead to early detection. However, whether early treatment (eg, anti-CD20 monoclonal antibody in case of a secondary EBV-positive B-cell lymphoma) will be effective after alemtuzumab-CHOP remains to be determined.

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**Authorship**

Contribution: H.C.K.-N. is the principal investigator of the HOVON 69 study, analyzed the data, and wrote the article; J.L.C. was responsible for case 2 and corrected the article; J.E.B. was responsible for case 2; G.W.v.I. is the coinvestigator of the HOVON 69 study, analyzed the data, and...
corrected the article; S.R. reviewed all pathology and corrected the article.

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References

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