

Unusual Skin Reactions after Mosquito Bites and Epstein-Barr Virus Reactivation in a Patient with Mantle Cell Lymphoma

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Abstract

We detected Epstein-Barr virus (EBV) reactivation in a patient with mantle cell lymphoma (MCL). The patient, a 53-year-old Japanese man, had been referred to our hospital because of generalized lymphadenopathy, hepatosplenomegaly and lymphocytosis and gave a history of intense skin reactions to mosquito bites. The biopsied lymph node contained a monotonous proliferation of medium-sized lymphocytes with scant cytoplasm and slightly irregular nuclei that were CD5⁺, CD20⁺ and CD23⁻. Antibody titers of IgG against EBV viral capsid antigen and early antigen were increased, and EBV was detected in the lymphoma cells. This case may suggest a relationship between EBV and MCL. (Internal Medicine 43: 986–989, 2004)

Key words: Chronic active Epstein-Barr virus infection, Chronic Epstein-Barr virus infection, Epstein-Barr virus, Epstein-Barr virus reactivation, hypersensitivity to mosquito bites, mantle cell lymphoma

Introduction

Hypersensitivity to mosquito bites (HMB) is a mysterious disorder that has been reported mainly in Japanese patients in the first two decades of life (1). The skin lesion at bite sites is typically a bulla that develops into necrosis. Patients simultaneously exhibit a high temperature and general malaise and subsequently may experience lymphadenopathy and

hepatosplenomegaly. Recent studies have revealed that this mosquito bite hypersensitivity is associated with chronic Epstein-Barr virus (EBV) infection and natural killer (NK) cell leukemia/lymphoma (2). Furthermore, Mori et al reported the first case of a patient with HMB who developed mantle cell lymphoma (MCL) (3). In their reports, the patient was a 61-year-old Japanese man. HMB was diagnosed based on the typical skin symptoms, namely bullae formation followed by ulcer and scar formation, and fever after mosquito bites. Biopsy of an inguinal lymph node showed a monotonous proliferation of lymphoma cells that were positive for CD5, CD19, CD20, surface membrane (sm)-IgM, and sm-κ. Antibody titers against EBV indicated chronic infection with this virus, and EBV was detected in the tumor cells in the lymph node. They speculated that EBV may have played some role in the development of MCL in this case. EBV is the etiologic agent of acute infectious mononucleosis and is closely associated with the genesis of Burkitt's lymphoma and undifferentiated nasopharyngeal carcinoma (4). EBV is also implicated in a variety of other diseases, such as EBV-associated hemophagocytic syndrome, Chronic active EBV infection (CAEBV), T-cell lymphoma, NK cell leukemia/lymphoma, lymphoproliferative diseases in immunocompromised hosts, Hodgkin's disease, pyothorax-associated B-cell lymphoma, smooth-muscle tumors, and gastric carcinoma. However, it has not been established that EBV is associated with MCL.

This paper shows EBV reactivation in a patient with MCL and a history of intense skin reactions to mosquito bites.

Case Report

A 53-year-old Japanese man was referred to our hospital

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Received for publication December 15, 2003; Accepted for publication June 3, 2004

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on February 6, 2002, for the evaluation of hepatosplenomegaly, generalized lymphadenopathy, lymphocytosis and thrombocytopenia. Family history was noncontributory. The patient had been well until three years previously, when he began to experience intense skin reactions to mosquito bites. Figure 1 shows a hemorrhagic bulla present on the sole of his foot after mosquito bites. Hematological examinations revealed a red blood cell count of $328 \times 10^4/\text{mm}^3$, hemoglobin level of 9.5 g/dl, white blood cell count of $30,800/\text{mm}^3$ with 87.8% lymphocytes and platelet count of $7.6 \times 10^4/\text{mm}^3$. The lymphocytes were mostly medium-sized and often had deeply clefted nuclei. Immunophenotypic analysis of lymphocytes was performed, with the following findings: 36.8% CD3⁺, 14.5% CD4⁺, 87.2% CD5⁺, 17.7% CD8⁺, 7.2% CD10⁺, 51.3% CD19⁺, 65.7% CD20⁺, 11.7% CD56⁺, and 78.4% HLA-DR⁺ cells. Cytogenetic examination of peripheral blood cells by fluorescence *in situ* hybridization (FISH) showed a chromosomal translocation, t(11;14)(q13;q32). On immunological examinations, the serum immunoglobulin levels were IgG 1,699 mg/dl, IgA 169 mg/dl, and IgM 37 mg/dl. His serum IgE level was increased to 55,000 IU/ml. Neither rheumatoid factor nor antinuclear antibody was present. The levels of serum EBV-specific antibodies were as follows: VCA-IgG, 1 : 2,560; VCA-IgA, 1 : 40; VCA-IgM, 1 : <10; EADR-IgG, 1 : 320; EADR-IgA, 1 : 40; EBNA, 1 : 40. Three hundred and ninety copies of EBV DNA per ml were detected in his serum. Antibodies to HIV-1 and HIV-2 were negative. Soluble interleukin 2 receptor was increased to 11,100 U/ml. Biopsy of a cervical lymph node showed a monotonous proliferation of medium-sized lymphocytes with scant cytoplasm and slightly irregular nuclei (Fig. 2). The lymphocytes were positive for CD5, CD19, CD20, CD21, CD22, FMC7, sm-IgM, sm-IgD, and sm- λ . They were negative for CD10 and CD23. Rearrangement of immunoglobulin heavy chain genes was detected in cervical lymph nodal cells by Southern blotting (data not shown). Based on these findings, the patient was diagnosed with MCL (5). Bone marrow biopsy also revealed a diffuse infiltration of lymphoma cells. Treatments with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone), ESHAP (etoposide, cisplatin, cytosine arabinoside, and methylprednisolone), and rituximab (a chimeric monoclonal antibody against the CD20 B-cell antigen) were not effective. Because of increasing splenomegaly and thrombocytopenia, he underwent splenectomy on October 11, 2002. The spleen was 3,200 g in weight and had a diffuse infiltrate of lymphoma cells. He then received 4 cycles of ESHAP plus rituximab and achieved partial remission. However, he showed progressive disease in April 2003. Combination chemotherapy of cladribine and mitoxantrone was administered without success (6). Treatment with CHASE (cyclophosphamide, cytosine arabinoside, etoposide, dexamethasone) was complicated by pneumonia and discontinued (7). He died of progressive disease on August 20, 2003. Permission for postmortem examination was not obtained.

Polymerase chain reaction (PCR) was performed to detect



Figure 1. Skin lesion at the bite site. A hemorrhagic bulla was present on the sole.

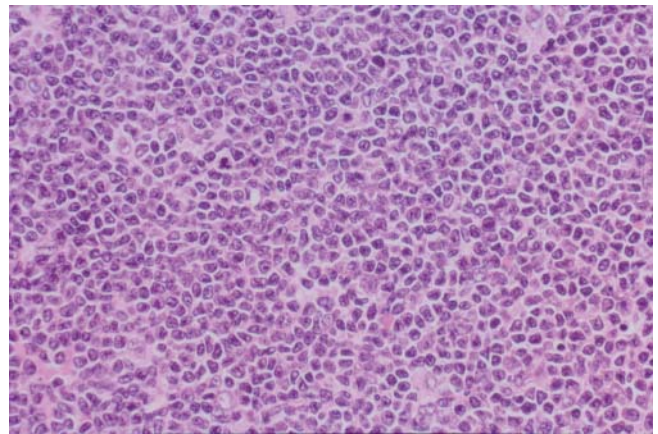


Figure 2. Lymph node section showing monotonous proliferation of medium-sized lymphocytes with slightly irregular nuclear contours (HE stain, $\times 140$).

EBV. DNA was extracted from the biopsied lymph node. The primers were designed to amplify an EBV-specific 161 base pair (bp) fragment in the *Bam* H1-W region (8). The primer sequences were as follows: F(sense) 5'-TCCTCGTC CAGCAAGAAGAG-3', R(anti-sense) 5'-CAACTTGAGG CAGCCTAATCC-3'). *In situ* hybridization (ISH) to detect EBV-RNA (EBER) was performed on paraffin-embedded samples using a commercial kit and probes (DakoCytomation A/S, Copenhagen, Denmark). EBV was detected in the lymph node by PCR (Fig. 3) and in the lymphoma cells by ISH (Fig. 4). Southern blotting of the DNA extracted from the biopsied lymph node with a probe, recognizing the termini of the EBV genome (9), demonstrated no band (data not shown).

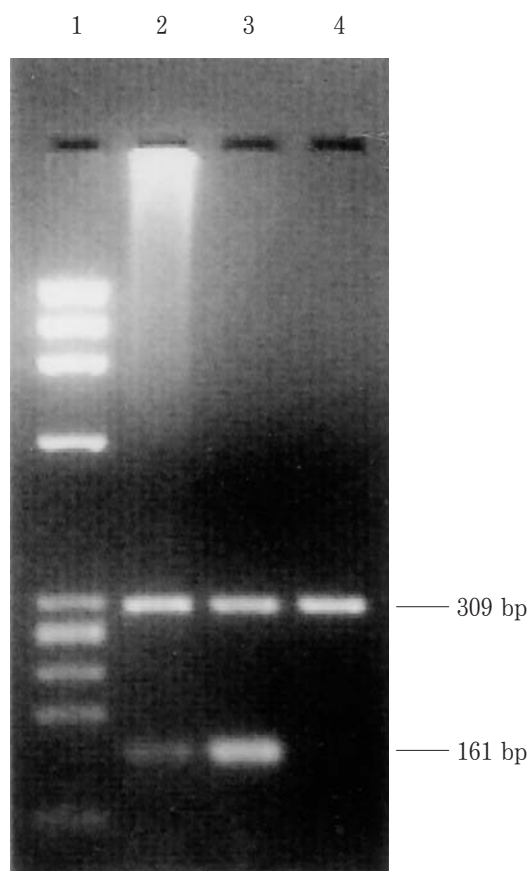


Figure 3. Detection of EBV DNA by PCR. PCR products were subjected to electrophoresis and stained with ethidium bromide. The 161-bp product was detected in the patient's biopsied lymph node. The 309-bp product was an internal control. Lane 1, molecular weight markers (ϕ X174 DNA/*Hae* III); Lane 2, patient (biopsied lymph node); Lane 3, positive control (Raji cells); Lane 4, negative control (peripheral blood mononuclear cells of a healthy volunteer).

Discussion

We detected EBV reactivation in a patient with MCL. His biopsied lymph node contained a monotonous proliferation of medium-sized lymphocytes with scant cytoplasm and slightly irregular nuclei. The lymphocytes were CD5⁺, CD10⁺, CD20⁺, CD23⁺, sm-IgM⁺ and sm-IgD⁺, with a chromosomal translocation, t(11;14)(q13;q32). These findings meet the criteria for the diagnosis of MCL (5). He gave a history of intense skin reactions to mosquito bites. His serum IgE level was markedly increased; however, since he lacked general symptoms such as high fever, he was not diagnosed with HMB (1). The pattern of his serum EBV-specific antibodies was compatible with reactivated or chronic infection with this virus (4), and EBV was detected in the lymphoma cells. However, he did not exhibit chronic or recurrent infectious mononucleosis-like symptoms lasting for a period of at

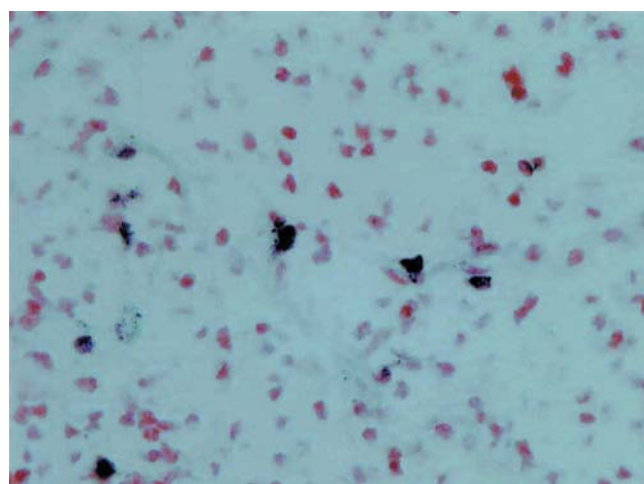


Figure 4. *In situ* hybridization for EBV-RNA (EBER). A paraffin-embedded lymph node was used as a sample. After being fixed on slides, the sample was hybridized with probes against EBER. Only a small proportion of cells were positive for EBER.

least 1 year, one of the diagnostic criteria for CAEBV (10). He was diagnosed with chronic EBV infection (1).

Hummel et al investigated 208 cases of B-cell non-Hodgkin's lymphoma (B-NHL) for their association with EBV infection (11). EBV was present in 54 cases of B-NHL. The virus was localized only in rare non-neoplastic bystander lymphocytes in 27 cases and additionally in tumor cells of 27 cases. The proportion of EBV-infected tumor cells present in the different cases varied between 1 and 100%. They suggested that in cases where EBV was present in 80–100% of tumor cells, EBV infection takes place before malignant transformation and thus may have contributed to the malignant phenotype, whereas in the other cases with only a smaller number of infected tumor and single bystander cells, EBV infection may have occurred following malignant transformation. In these cases, infection appeared to be of little or no significance in tumorigenesis. On the other hand, Daibata et al suggested that EBV infection, if it occurred in neoplastic lymphoma cells, could play a role in acquisition of malignant phenotypes (12, 13). They established EBV-positive (SP-50B) and EBV-negative (SP-53) cell lines with the t(11;14)(q13;q32) chromosome abnormality from a single patient with MCL. Only EBV-positive SP-50B cells possessed malignant phenotypes, such as growth ability in low serum, colony formation in soft agarose, and tumorigenicity in nude mice. A lymphoblastoid B-cell line established by infecting the patient's normal B lymphocytes *in vitro* with exogenous EBV had no tumorigenicity. Since in the present patient some scattered lymphoma cells showed an EBV presence, EBV infection may have occurred following malignant transformation, and the virus may have played some role in the development of MCL. In conclusion, our

case may suggest a relationship between EBV and MCL.

Acknowledgements: The authors thank Dr. Takanori Shikiji for the photo of the patient's skin lesion.

References

- 1) Tokura Y, Ishihara S, Tagawa S, Seo N, Ohshima K, Takigawa M. Hypersensitivity to mosquito bites as the primary clinical manifestation of a juvenile type of Epstein-Barr virus-associated natural killer cell leukemia/lymphoma. *J Am Acad Dermatol* **45**: 569–578, 2001.
- 2) Ishihara S, Okada S, Wakiguchi H, Kurashige T, Hirai K, Kawa-Ha K. Clonal lymphoproliferation following chronic active Epstein-Barr virus infection and hypersensitivity to mosquito bites. *Am J Hematol* **54**: 276–281, 1997.
- 3) Mori T, Okamoto S, Kuramochi S, Ikeda Y. An adult patient with hypersensitivity to mosquito bites developing mantle cell lymphoma. *Int J Hematol* **71**: 259–262, 2000.
- 4) Kawa K. Epstein-Barr virus-associated diseases in humans. *Int J Hematol* **71**: 108–117, 2000.
- 5) Zucca E, Stein H, Coiffier B. European lymphoma task force (ELTF): report of the workshop on mantle cell lymphoma (MCL). *Ann Oncol* **5**: 507–511, 1994.
- 6) Rummel MJ, Chow KU, Karakas T, et al. Reduced-dose cladribine (2-CdA) plus mitoxantrone is effective in the treatment of mantle-cell and low-grade non-Hodgkin's lymphoma. *Eur J Cancer* **38**: 1739–1746, 2002.
- 7) Ogura M, Kagami Y, Taji H, et al. Pilot phase I/II study of new salvage therapy (CHASE) for refractory or relapsed malignant lymphoma. *Int J Hematol* **77**: 503–511, 2003.
- 8) Saito I, Servenius B, Compton T, Fox RI. Detection of Epstein-Barr virus DNA by polymerase chain reaction in blood and tissue biopsies from patients with Sjögren's syndrome. *J Exp Med* **169**: 2191–2198, 1989.
- 9) Raab-Traub N, Flynn K. The structure of the termini of the Epstein-Barr virus as a marker of clonal cellular proliferation. *Cell* **47**: 883–889, 1986.
- 10) Straus SE. The chronic mononucleosis syndrome. *J Infect Dis* **157**: 405–412, 1988.
- 11) Hummel M, Anagnostopoulos I, Korbjuhn P, Stein H. Epstein-Barr virus in B-cell non-Hodgkin's lymphomas: Unexpected infection patterns and different infection incidence in low-and high-grade types. *J Pathol* **175**: 263–271, 1995.
- 12) Daibata M, Kubonishi I, Eguchi T, Yano S, Ohtsuki Y, Miyoshi I. The establishment of Epstein-Barr virus nuclear antigen-positive (SP-50B) and Epstein-Barr virus nuclear antigen-negative (SP-53) cell lines with t(11;14)(q13;q32) chromosome abnormality from an intermediate lymphocytic lymphoma. *Cancer* **64**: 1248–1253, 1989.
- 13) Daibata M, Kubonishi I, Miyoshi I. Differential tumorigenicity between Epstein-Barr virus genome-positive and genome-negative cell lines with t(11;14)(q13;q32) derived from mantle cell lymphoma. *J Virol* **70**: 9003–9007, 1996.