J Clin Exp Hematopathol
Vol. 50, No. 1, May 2010

Case Study

Small Bowel Perforation Caused by Epstein-Barr Virus-Associated B Cell Lymphoma in a Patient with Angioimmunoblastic T-Cell Lymphoma

Tsutomu Takahashi,[1)] Riruke Maruyama,[2)] Satoko Mishima,[3)] Masaya Inoue,[4)] Koshi Kawakami,[1)] Chie Onishi,[1)] Takaaki Miyake,[1)] Junko Tanaka,[1)] Toru Nabika,[3)] and Hiroto Ishikura[4)]

On rare occasions, secondary Epstein-Barr virus (EBV)-associated B cell lymphoma can develop in a patient with angioimmunoblastic T-cell lymphoma (AITL). We report a case of a 66-year-old Japanese woman who developed diffuse large B-cell lymphoma (DLBCL) in her small intestine after chemotherapy for AITL. She was found to have panperitonitis due to perforation of the small intestine. Partial ileectomy specimen showed DLBCL cells infiltrating into the intestinal wall. *In situ* hybridization for EBV-encoded RNA revealed positivity in the lymphoma cells. The lymph nodes diagnosed as AITL were negative for EBV infection and there was no coexistence of B cell neoplasms in them. We thought small bowel perforation in this case was caused by EBV-associated B cell lymphoma secondary to AITL. Our case showed a remarkable deficiency of cellular immunity after chemotherapy, which we postulate was related to the cause of occurrence of B-cell lymphoma. 〔*J Clin Exp Hematopathol 50(1) : 59-63, 2010*〕

Keywords: angioimmunoblastic T-cell lymphoma, small bowel perforation, Epstein-Barr virus, secondary B cell lymphoma

INTRODUCTION

Angioimmunoblastic T-cell lymphoma (AITL) is one of the nodal peripheral T-cell lymphomas described in the WHO classification that accounts for 4-6% and 5% of all lymphomas in Western countries[1-3] and in Japan,[4] respectively.

AITL typically presents with advanced stage disease, and is manifested by generalized lymphadenopathy, hepatosplenomegaly, and other systemic symptoms. Patients exhibit immunodeficiency secondary to the neoplastic process.[1-3] In the majority of cases, expansion of B-cells positive for Epstein-Barr virus (EBV) is seen, which is thought to be a consequence of underlying immune dysfunction.[5,6]

It has been reported that supervening EBV-associated B cell lymphoma can occur in AITL. However, relatively few cases of EBV-associated B cell lymphomas in AITL have been fully described so far.[7-15] In a previous large study, only three cases out of more than 170 cases with AITL developed EBV-associated secondary B cell lymphomas.[10]

We report here a rare case of AITL, in which small bowel perforation was thought to be caused by EBV-associated B cell lymphoma after combination chemotherapy and high dose corticosteroid treatment, and discuss the etiology of the secondary B cell lymphoma with a review of the literature.

Received : June 25, 2009
Revised : July 27, 2009
Accepted : August 12, 2009

[1)]Department of Hematology,[2)] Department of Organ Pathology and [3)]Department of Functional Pathology, Shimane University Faculty of Medicine, and [4)]Division of Clinical Study of Oncology, Shimane University Hospital, 89-1 Enya-chou, Izumo-shi, Shimane 693-8501, Japan.
Address correspondence and reprint request to Hiroto Ishikura, M.D., Division of Clinical Study of Oncology, Shimane University Hospital, 89-1 Enya-chou, Izumo-shi, Shimane 693-8501, Japan.
E-mail : ben2106t@med.shimane-u.ac.jp

CASE REPORT

In February 2005, a 66-year-old woman was admitted to our hospital due to continuous fever and loss of body weight. Clinical examination revealed general lymphadenopathy, splenomegaly, pretibial edema and purpura. Laboratory findings showed increase of the white blood cell count, serum lactate dehydrogenase, serum C-reactive protein and hypoalbuminemia. Eosinophilia and hyper-γ-globulinemia were not observed. Tests for viral capsid antigen (VCA)-IgG and EBV-associated antigen were positive but VCA-IgM was negative. Anti-human immunodeficiency virus antibody and anti-human T-cell lymphotropic virus-1 antibody were negative. Computed tomography (CT) showed multiple lymph node swellings, bilateral pleural effusion, pericardial effusion and hepatosplenomegaly. An evaluation of bone marrow aspirate showed no infiltration of atypical cells. No EBV-

infected cells were found by small EBV-encoded RNA *in situ* hybridization (EBER-ISH) in bone marrow.

The patient then underwent biopsy of the left inguinal lymph node. Hematoxylin-eosin stained sections showed an effacement of normal nodal architecture by polymorphic cellular infiltration, which was seen beyond the capsule of the lymph node. Diffuse infiltration was composed of small to medium-sized or large lymphoid cells admixed with plasma cells and eosinophils. Some of the large immunoblast-like lymphoid cells had a clear cytoplasm. There was also a proliferation of high-endothelial venules. Immunohistochemical staining revealed that the large immunoblast-like lymphoid cells were predominantly positive for CD3, CD10 and CXCL13. Southern blot analysis detected clonal rearrangement of the *T-cell receptor (TCR) Cβ1* gene, but clonal rearrangement of the *immunoglobulin heavy chain* (*IgH*) gene was not demonstrated. These findings were compatible with AITL. EBER-ISH showed negativity in the lymphoma cells as well as in the reactive small lymphocytes (Fig. 1).

The patient received one cycle of THPCOP[16] (cyclophosphamide, pirarubicin, vincristine and prednisolone), but her symptoms did not improve. She received CHASE[17] (cyclophosphamide, cytarabine, etoposide and dexamethasone) as a salvage therapy. Her symptoms improved after two cycles of CHASE, but pancytopenia persisted. She could not continue to receive further chemotherapy. Bone marrow aspiration revealed hypocellularity and gelatinous change of the stroma. There was no neoplastic cell infiltration, hemophagocytosis, or EBV-infected cells. She was followed-up without any further treatment for one year. During this follow-up period, pancytopenia and suppression of cellular immunity was observed (Mean absolute count of CD3 positive lymphocytes was 238/μL, CD4 positive lymphocytes was 114/μL, CD8 positive lymphocytes was 131/μL). A slight increase in cytomegalovirus (CMV) antigenemia was observed without clinical symptoms.

In September 2006, the patient developed disseminated varicella-zoster followed by high grade fever, generalized lymphadenopathy and splenomegaly. A biopsy of axillary lymph node revealed a relapse of AITL. At this point, EBV positive B immunoblasts did not coexist. Since pancytopenia had persisted, she received high-dose dexamethasone therapy. Lymph node swellings completely disappeared, but pancytopenia was worsening. Neoplastic cell infiltration and EBV-infected cells into bone marrow was not observed. She was afebrile and doing well during the following two months.

In January 2007, she presented with high fever again. We suspected the second relapse of AITL and employed treatment by prednisolone. However, a significant increase in CMV antigenemia was demonstrated and CT showed neither lymph node swellings nor splenomegaly, although an increase in the intestinal gas, a thickening of the intestinal wall and an appearance of ascites were noticed. She was diagnosed as having CMV colitis and the intravenous administration of ganciclovir was started. Twelve days later, she suddenly complained of severe abdominal pain because of intestinal perforation. Partial resection of the ileum and colostomy were performed urgently. Macroscopically, the resected terminal ileum showed a short segment of prominent hemorrhagic necrosis. The mucosa was dark and granular in appearance with three pinhole perforation sites. Microscopic examination showed a diffuse infiltration of atypical large lymphoid cells in the whole thickness of the intestinal wall with hemorrhagic and necrotic changes. Immunohistochemical staining revealed that the large atypical cells were positive for CD20 and CD79a, but negative for CD3 and CD45RO. PCR analysis showed monoclonal rearrangement of the *IgH* gene, but no clonal rearrangement of the *TCR-γ* gene was detected. A diagnosis of diffuse large B cell lymphoma was made. Some lymphoma cells were positive for latent membrane protein-1, but EBV-associated antigen-2 was not expressed in both of the lymphoma cells and reactive small lymphocytes. EBER-ISH showed positivity in both cells (Fig. 2). Operative findings and pathological examination revealed no evidence of vascular occlusion. We considered the perforation to have been caused by lymphoma cell infiltration. After the surgery, high grade fever continued and CT revealed persistent retention of ascites and multiple space-occupying lesions in the liver. Needle biopsy of the liver did not show infiltrating neoplastic cells but it did reveal a fraction of necrotic tissue. Simultaneously, the patient complained of visual disturbance, and tumor involvement of the fundus was suspected based on an ophthalmological examination.

In March 2007, she died of respiratory failure caused by severe pneumonia. The family refused an autopsy.

DISCUSSION

The lymphoma cells present in the intestinal wall in our case were thought to be derived from EBV-infected B cells because of the positivity for ISH-EBER and positive immunohistochemical reaction for latent membrane protein-1. It is well known that most cases of AITL are frequently complicated with EBV infections. It has been reported that EBV genomes are found in most of the AITL cases[5] and almost all of the cells infected by EBV are B cells showing normal histological findings.[18] In the majority of cases, EBER-positive cells were scattered, but there were sheets of EBV-positive cells in some cases.[19] In spite of such a background, the complication of B cell lymphomas in AITL has been reported to be rare. Zettl *et al*.[10] investigated more than 600 cases of nodal T-cell lymphoma including more than 170 cases of AITL. They found 17 cases with transformed large EBV-positive B cells and only three of these cases were secondary EBV-associated B cell lymphoma in AITL. To the best of our knowledge, eighteen cases of AITL with secon-

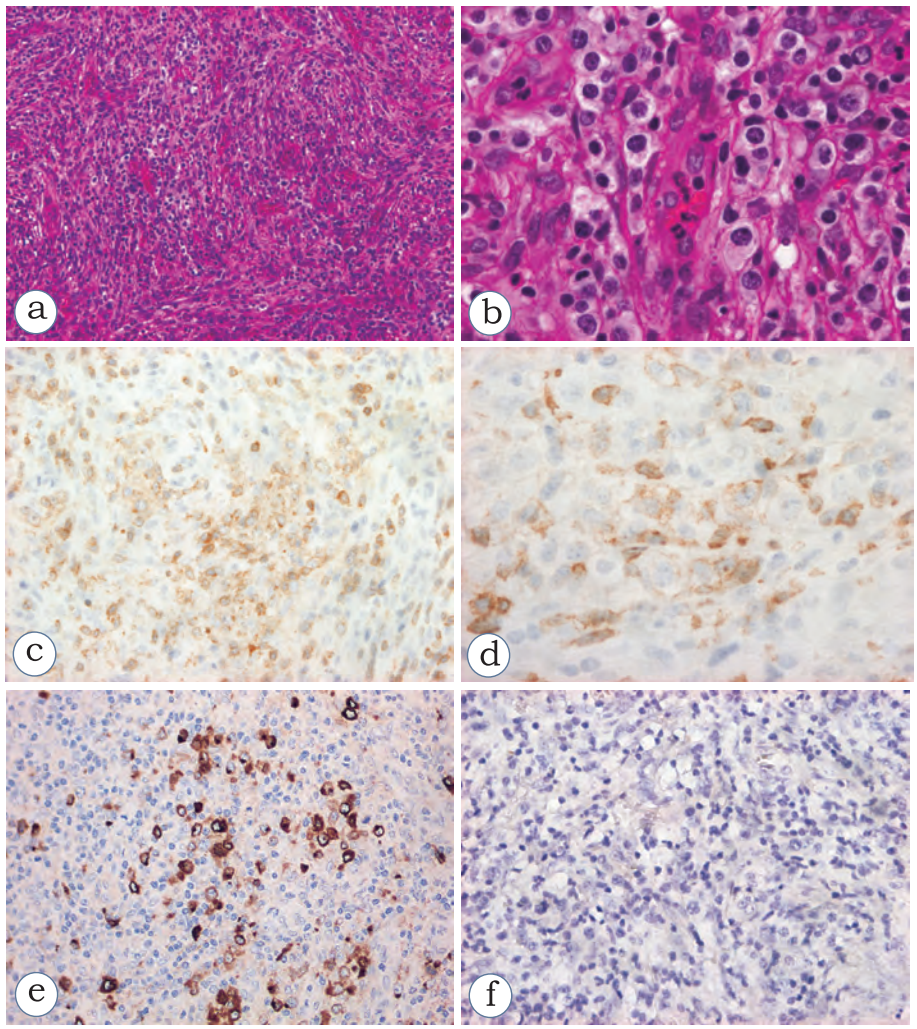

Fig. 1. Microscopic appearances of the lymph node at the initial presentation. (***1a***) The normal architecture was effaced by a diffuse polymorphic cellular infiltrate. H&E stain, ×100. (***1b***) The diffuse infiltration is composed of a variety of infiltrating cells, including immunoblast-like large cells with clear cytoplasm around the high-endothelial venules. H&E stain, ×400. (***1c***)-(***1e***) Immunohistochemical examination of the lymph node at presentation. The clear immunoblast-like cells are positively stained with CD3 (*1c*), CD10 (*1d*) and CXCR13 (*1e*). Counterstained with hematoxylin, (*1c*) & (*1e*) ×200, (*1d*) ×400. (***1f***) Epstein-Barr virus-encoded RNA *in situ* hybridization showed negativity in the lymphoma cells as well as in the reactive small lymphocytes. Counterstained with hematoxylin, ×200.

dary EBV-associated B cell lymphoma have been previously reported.[7-15]

It is commonly considered that EBV-associated secondary B cell lymphomas in AITL develop from EBV-infected B cells in lymph nodes. However, EBV-infected B cells were not detected by EBER-ISH before development of secondary lymphoma in our case. This fact drove us to review the literature, and we found that in five out of nine cases investigated by EBER-ISH at initial presentation of AITL, EBV-infected cells were not detected.[7-11,13] Furthermore, these cases as well as ours presented extranodal organ involvement by secondary B-cell lymphomas. We thus conclude that secondary lymphomas could occur from anywhere other than a lymph node of AITL.

This immunosuppression is thought to be related to occurrence of EBV-associated disease conditions such as post-transplant lymphoproliferative disorders,[20] lymphomas associated with human immunodeficiency virus infection,[21] or age-

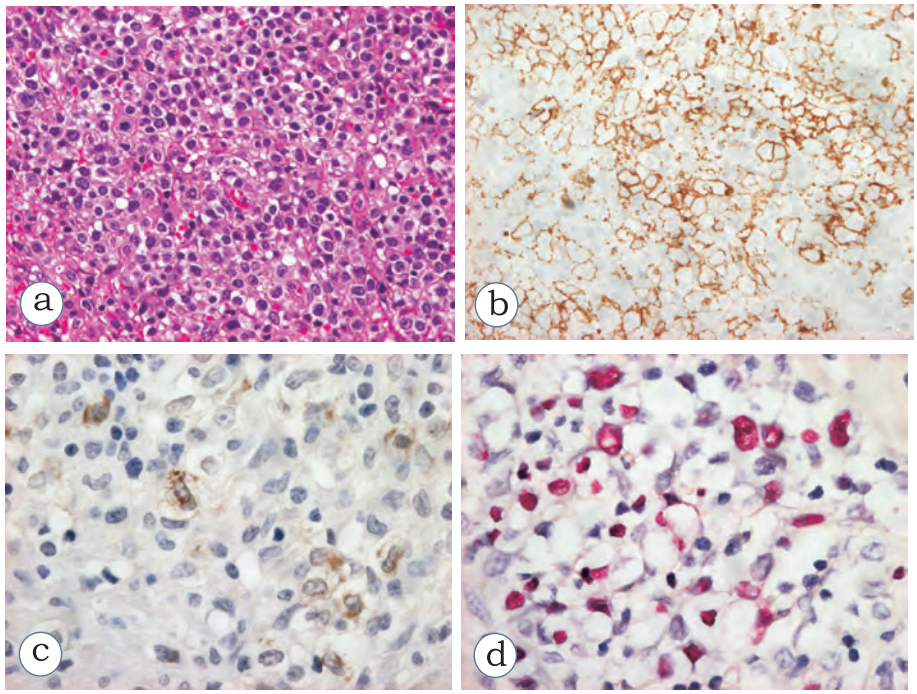

Fig. 2. Pathological findings of diffuse large B-cell lymphoma proliferating in the ileal wall. (***2a***) Large atypical lymphoid cells with prominent nucleoli are seen in the hemorrhagic necrotizing wall of the ileum. H&E stain, ×200. (***2b***) Atypical large cells are stained positively with CD20. Counterstained with hematoxylin, ×200. (***2c***) Latent membrane protein-1 is also positive in some of them. Counterstained with hematoxylin, ×400. (***2d***) Epstein-Barr virus-encoded RNA *in situ* hybridization shows positivity in both of lymphoma cells and reactive small lymphocytes. Counterstained with hematoxylin, ×400.

related EBV-associated lymphoproliferative disorders.[22] It is well known that patients with AITL tend to be severely immunodeficient. Moreover, a recent study has revealed that an increase in EBV load in lymph nodes is associated with histological progression of AITL.[23] However, because the occurrence of secondary EBV-associated B cell lymphoma is rare, it is considered that secondary lymphomas are induced not only by immunodeficiency of AITL, but also by additional factors such as treatment and patient's background. Recently, Weisel *et al*.[15] reported a case where EBV-associated secondary B cell lymphoma developed after chemotherapy composed of alemtuzumab and fludarabine. Their case showed remarkable a deficiency of cellular immunity, as was seen in our case. Our case showed persistent pancytopenia after chemotherapy, deficiency of cellular immunity and reactivation of CMV and varicella zoster virus, which indicated a deeply immunodeficient state. We consider the immunodeficiency to probably be related to lymphomagenesis in our case. However, the reason for remarkable immunodeficiency after chemotherapy in our case is not exactly clear. In the previous reports, the information about treatment was described in twelve cases.[7-11,13-15] In these reports, four cases underwent autologous stem cell transplantation and on the other hand, two cases developed secondary lymphoma after observation alone. Therefore, it is still unclear whether treatment is related to lymphomagenesis.

In conclusion, we observed an unexpected bowel perforation that was caused by EBV-associated secondary B cell lymphoma in AITL. Clinicians should keep in mind that there is a possibility of this phenomenon, particularly when a deep cellular immunosuppressive state is observed. It is critical that further investigations, together with the accumulation of such cases be conducted, so that we can better understand the clinicopathological features of patients with this kind of complicated lymphoma.

ACKNOWLEDGEMENTS

The authors thank Prof. Koichi Ohshima in Department of Pathology, School of Medicine, Kurume University for technical assistance.

REFERENCES

1 Dogan A, Gaulard P, Jaffe ES, Ralfkiaer E, Müller-Hermelink HK : Angioimmunoblastic T cell lymphoma. In : Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, *et al.* (eds) : World Health Organization Classification of Tumours, WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed, International Agency for Research on Cancer (IARC), Lyon, pp. 309-311, 2008

2 Rizvi MA, Evens AM, Tallman MS, Nelson BP, Rosen ST : T-cell non-Hodgkin lymphoma. Blood 107 : 1255-1264, 2006

3 Dogan A, Attygalle AD, Kyriakou C : Angioimmunoblastic T-cell lymphoma. Br J Haematol 121 : 681-691, 2003

4 Aoki R, Karube K, Sugita Y, Nomura Y, Shimizu K, *et al.* : Distribution of malignant lymphoma in Japan : Analysis of 2260 cases, 2001-2006. Pathol Int 58 : 174-182, 2008

5 Weiss LM, Jaffe ES, Liu XF, Chen YY, Shibata D, *et al.* : Detection and localization of Epstein-Barr viral genomes in angioimmunoblastic lymphadenopathy and angioimmunoblastic lymphadenopathy-like lymphoma. Blood 79 : 1789-1795, 1992

6 Attygalle AD, Chuang SS, Diss TC, Du MQ, Isaacson PG, *et al.* : Distinguishing angioimmunoblastic T-cell lymphoma from peripheral T-cell lymphoma, unspecified, using morphology, immunophenotype and molecular genetics. Histopathology 50 : 498-508, 2007

7 Abruzzo LV, Schmidt K, Weiss LM, Jaffe ES, Medeiros LJ, *et al.* : B-cell lymphoma after angioimmunoblastic lymphadenopathy : a case with oligoclonal gene rearrangements associated with Epstein-Barr virus. Blood 82 : 241-246, 1993

8 Knecht H, Martius F, Bachmann E, Hoffman T, Zimmermann DR, *et al.* : A deletion mutant of the LMP1 oncogene of Epstein-Barr virus is associated with evolution of angioimmunoblastic lymphadenopathy into B immunoblastic lymphoma. Leukemia 9 : 458-465, 1995

9 Matsue K, Itoh M, Tsukuda K, Kokubo T, Hirose Y : Development of Epstein-Barr virus-associated B cell lymphoma after intensive treatment of patients with angioimmunoblastic lymphadenopathy with dysproteinemia. Int J Hematol 67 : 319-329, 1998

10 Zettl A, Lee SS, Rüdiger T, Starostik P, Marino M, *et al.* : Epstein-Barr virus-associated B-cell lymphoproliferative disorders in angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphoma, unspecified. Am J Clin Pathol 117 : 368-379, 2002

11 Attygalle AD, Kyriakou C, Dupuis J, Grogg KL, Diss TC, *et al.* : Histologic evolution of angioimmunoblastic T-cell lymphoma in consecutive biopsies : clinical correlation and insights into natural history and disease progression. Am J Surg Pathol 31 : 1077-1088, 2007

12 Willenbrock K, Bräuninger A, Hansmann ML : Frequent occurrence of B-cell lymphomas in angioimmunoblastic T-cell lymphoma and proliferation of Epstein-Barr virus-infected cells in early cases. Br J Haematol 138 : 733-739, 2007

13 Hawley RC, Cankovic M, Zarbo RJ : Angioimmunoblastic T-cell lymphoma with supervening Epstein-Barr virus-associated large B-cell lymphoma. Arch Pathol Lab Med 130 : 1707-1711, 2006

14 Park S, Noguera ME, Brière J, Feuillard J, Cayuela JM, *et al.* : Successful rituximab treatment of an EBV-related lymphoproliferative disease arising after autologous transplantation for angioimmunoblastic T-cell lymphoma. Hematol J 3 : 317-320, 2002

15 Weisel KC, Weidmann E, Anagnostopoulos I, Kanz L, Pezzutto A, *et al.* : Epstein-Barr virus-associated B-cell lymphoma secondary to FCD-C therapy in patients with peripheral T-cell lymphoma. Int J Hematol 88 : 434-440, 2008

16 Kitamura K, Takaku F : Pirarubicin, a novel derivative of doxorubicin. THP-COP therapy for non-Hodgkin's lymphoma in the elderly. Am J Clin Oncol 13 (Suppl 1) : S15-19, 1990

17 Ogura M, Kagami Y, Taji H, Suzuki R, Miura K, *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

18 Bräuninger A, Spieker T, Willenbrock K, Gaulard P, Wacker HH, *et al.* : Survival and clonal expansion of mutating "forbidden" (immunoglobulin receptor-deficient) Epstein-Barr virus-infected B cells in angioimmunoblastic T cell lymphoma. J Exp Med 194 : 927-940, 2001

19 Mourad N, Mounier N, Brière J, Raffoux E, Delmer A, *et al.* : Clinical, biologic, and pathologic features in 157 patients with angioimmunoblastic T-cell lymphoma treated within the Groupe d'Etude des Lymphomes de l'Adulte (GELA) trials. Blood 111 : 4463-4470, 2008

20 Swerdlow SH, Webber SA, Chadburn A, Ferry JA : Post-transplant lymphoproliferative disorders. In : Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, *et al.* (eds) : World Health Organization Classification of Tumours, WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed, International Agency for Research on Cancer (IARC), Lyon, pp. 343-349, 2008

21 Raphaël M, Said J, Borisch B, Cesarman E, Harris NL : Lymphomas associated with HIV infection. In : Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, *et al.* (eds) : World Health Organization Classification of Tumours, WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed, International Agency for Research on Cancer (IARC), Lyon, pp. 340-342, 2008

22 Shimoyama Y, Yamamoto K, Asano N, Oyama T, Kinoshita T, *et al.* : Age-related Epstein-Barr virus-associated B-cell lymphoproliferative disorders : special references to lymphomas surrounding this newly recognized clinicopathologic disease. Cancer Sci 99 : 1085-1091, 2008

23 Zhou Y, Attygalle AD, Chuang SS, Diss T, Ye H, *et al.* : Angioimmunoblastic T-cell lymphoma : histological progression associates with EBV and HHV6B viral load. Br J Haematol 138 : 44-53, 2007