Case report

Epstein–Barr virus load correlating with clinical manifestation and treatment response in a patient with angioimmunoblastic T-cell lymphoma

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Epstein–Barr virus (EBV)-associated lymphoma may arise secondary to angioimmunoblastic T-cell lymphoma (AITL). The prognosis is poor despite chemotherapy and experimental therapies. We report on a 40-year-old woman with AITL without obvious immunodeficiency in which EBV-associated lymphoma developed. The occurrence and size of enlarged lymph nodes correlated strongly with the EBV load in serum (EBVL). Treatment with valacyclovir at the early stage resulted in a drastic more than 3 log10 decrease of EBVL and complete remission. However, valacyclovir had to be stopped after 6 months due to side effects, and the lymphoma reoccurred 3 months later associated with increasing EBVL. Eventually started cytotoxic chemo- and anti-CD20 therapy resulted only in partial remission. The lymphoma progressed and 33 months after it was diagnosed the patient died. This case report demonstrates the close association of EBVL and AITL and a beneficial effect of antiviral therapy at an initial stage of disease manifestation.

Introduction

Angioimmunoblastic T-cell lymphoma (AITL), earlier described as an abnormal immune reaction, that is, angioimmunoblastic lymphadenopathy with dysproteinenaemia (AILD), was classified by the WHO as a peripheral T-cell lymphoma [1]. AITL is characterized clinically with constitutional symptoms, generalized lymphadenopathy, hepatosplenomegaly, skin rash and polyclonal hypergammaglobulinaemia. B-cell proliferations, particularly those related to Epstein–Barr virus (EBV) are thought to be secondary to immune dysregulation produced by AITL [2–4]. To note, EBV plays an important role in the pathogenesis of different lymphoproliferative disorders, in particular in the post-transplant setting [5–8]. The diagnosis of AITL is often difficult because of a varying clinical and pathological picture. Regarding prognosis only a quarter of patients experience long-term remissions even after multi-agent chemotherapy. In a series of 33 patients receiving CHOP-like regimens (cyclophosphamide, doxorubicin, vincristine, prednisone) for a median number of six cycles a median survival of 36 months and an overall survival at 5 years of 36% were reported [3]. This and smaller case series document that AITL has a poorer prognosis compared to other non-Hodgkin’s lymphomas [4]. Complete, temporary remissions have been reported after the use of experimental therapeutic modalities including interferon-α, cyclosporin A, and recently purine analogues such as fludarabine [9,10].

Here we report on an immunocompetent woman in whom AITL disease occurrence and degree of lymph node enlargement strongly correlated with EBV load (EBVL) in the serum. To the best of our knowledge, this is the first case to demonstrate clearly an association of the degree of EBVL with disease manifestation of AITL and a beneficial effect of antiviral therapy in AITL at an initial stage.
Case history

This 40-year-old woman reported painful swelling of bilateral lymph nodes at the neck, axilla and inguina in February 2000. Concomitantly, she suffered from fatigue, headache and initially intermittent night sweats, but had no fever or weight loss. Her personal history was unremarkable except for a thyroid goitre diagnosed in 1991 and treated with neomercazole and inderal until mid-1999.

In May 2000, she presented with severe dyspnea and multiple extremely painful enlarged lymph nodes located bilaterally at the neck (size 7×8 cm), supraclavicular fossa, axilla and inguina (diameter 3–4 cm). Pain was relieved by administration of diclofenac. The chest X-ray revealed hilar enlargement and partial tracheal compression. The abdominal computed tomography showed two para-aortic hyperechogenic lymph node-like structures measuring 1.3 and 2.3 cm, respectively. The white blood cell count (6.52×10⁹/l) was not elevated and showed normal differentiation. Other relevant values were within normal limits (alanine transaminase, creatinine, electrolytes) except for an elevated lactate dehydrogenase activity of 819 U/l (reference 232–437 U/l). Immunochemistry showed a slightly elevated total protein (84 g/l) and a slight hypergammaglobulinaemia (17.2 g%). Immunoglobulin (Ig)G to the EBV early antigen (EA; Gull Laboratories, Salt Lake City, USA), viral capsid antigen (VCA; Merifluor) and the nuclear antigen (NA; Focus Technologies, Herndon, USA) were positive, but no IgM to EA or VCA were detectable.

Enlarged mediastinal lymph nodes were surgically removed to relieve tracheal compression. Histology revealed AITL with proliferating vessels and showed a clonal rearrangement of T-cell receptors (TCR) and Ig genes could not be demonstrated. Other infectious aetiologies such as tuberculosis were excluded by culture and specific polymerase chain reaction (PCR). To rule out uncommon bacteria eubacterial PCR was used. Briefly, culture-independent molecular techniques involving the use of broad-range PCR amplification of a part of the 16S rRNA bacterial gene followed by single-strand sequencing were applied [13].

The enlarged cervical lymph nodes fluctuated somewhat (approximately ±30%) in size, but progression to severe disease with fatigue and severe generalized lymphadenopathy was noted early in September 2000. Thoracal and abdominal computed tomography showed enlarged lymph nodes at different sites (Figure 1). Histology of a lymph node from the axilla confirmed AITL with destroyed lymph node architecture lacking germinal centres. Also, other typical features of AITL, earlier defined as angioimmunoblastic lymphadenopathy (AILD) were confirmed [2]. Upon staining, large immunoblasts showed expression of CD3, CD20 and CD30. Ki67 staining of 70% of these immunoblasts documented a high proliferation rate. EBV was demonstrated by in situ hybridization showing a nuclear positivity for small EBV encoded nuclear RNA (EBER-1) and cytoplasmic positivity for latent membrane protein (LMP) (Figure 2A–C). TCR-γ rearrangement was examined with semi-nested multiplex PCR for variable TCR-γ genes in subgroups V-I, V-II, V-III, V-IV and for TCR-γ joint genes J-1, J-2, JP, JP-1, JP-2. These examinations in September 2000 demonstrated a polyclonal pattern without a sign of clonal TCR-γ rearrangement. Immunoglobulin heavy chain rearrangements were examined by PCR for the framework region 1 and 3, respectively, of variable IgH genes and for IgH joint genes. Investigations of two independent probes of framework region 1 and 3, respectively, showed a clonal IgH rearrangement indicating a clonal B-cell population. In comparison to the prior biopsy an increase of blasts was demonstrated in addition to the new clonal population. The patient showed no obvious immunodeficiency as assessed by measurements of CD4 cell count and immunoglobulins. Repeated HIV tests were negative.

The cellular immune reactivity against EBV antigens was assessed by determining the frequency of EBV-specific cytotoxic T lymphocyte precursors against different viral epitopes in peripheral blood mononuclear cells (PBMCs) using limiting dilution analysis following stimulation with an autologous EBV-immortalized lymphoblastoid cell line [14]. The EBV-specific
Figure 2. Lymph node histology and EBV-specific stainings

(A) Lymph node (September 2000) lacking a clear follicular structure. PAS (periodic acid Schiff) positivity in walls of vessel which shows arborisating proliferation and immunoblasts (arrow); (B) Epstein–Barr virus encoded RNA-1 (EBER) staining indicates significant nuclear positivity in lymphoid cells (arrow) of a lymph node section; (C) Epstein-Barr virus latent membrane protein-1 (LMP) staining demonstrating strong cytoplasmic positivity (arrow) in a lymph node section.
cytotoxic T-cell precursor frequency was determined as 16 per million PBMCs, thus indicating the presence of a functional, albeit low cytotoxic T-cell response against EBV.

EBVL was monitored closely by real-time PCR [15,16]. Quantitative DNA analysis was performed using the TaqMan® (Applied Biosystems, Rotkreuz, Switzerland) real-time PCR technique. In addition to the two PCR amplimers, this assay also uses a fluorescent resonance energy transfer hybridization probe. PCR primers and a hybridization probe were selected for the amplification of a 101 bp sequence in the conserved BamHI W region of the EBV genome, as reported [15]. Briefly, DNA was extracted from 1 ml serum after passage through a 0.45 µm filter and addition of salmon sperm carrier DNA. PCR reactions were run with 40 cycles of 15 s 95°C and 60 s in an ABI prism 7700 sequence detector using the TaqMan 2× Universal Master Mix (Perkin Elmer) with 300 nM of each primer and 200 nM of the fluorescent TaqMan probe. Each sample was analysed in triplicate, positive and negative controls were included in every run. A pUC18 plasmid containing the EBV BamHI W fragment included in every PCR run served as internal control and calibration curve and allowed to quantify the EBV DNA copies.

As shown in Figure 3, EBV DNA was detectable in serum, although at a low level (73 copies/ml) in February 2000, but increased markedly when the patient presented with enlarged lymph nodes in May 2000. Peak EBVL were found (134 000 copies/ml = 5.13 log_{10}) when the patient was hospitalized with severe lymph node enlargement and dyspnea due to lymphomular compression of the trachea in September 2000 (Figure 3). Intravenous acyclovir was considered at that time, however, the patient declined hospitalization for a prolonged time. Treatment (9.9.00) with valacyclovir (1 g three times daily) resulted in a dramatic decrease of EBVL to levels of 26 copies/ml (~3.69 log_{10} as of peak and 3.00 log_{10} as of start of antiviral therapy). Within 1 week, lymph nodes that measured between 3 and 8 cm in diameter showed a subtotal or a total regression (decrease in size to <1 cm). In parallel, fatigue and night sweats disappeared and the patient felt well again. During this time, all laboratory parameters that were pathological in early September normalized including alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, albumin and globulins. Due to increasing nausea despite antinauseants, the occurrence of oedema and weight gain of 12 kg, an adverse response to valacyclovir was suspected, and the dose was lowered to 1 g/day and after a total period of 6 months eventually discontinued (Figure 3). Oedema vanished but lymph node enlargement reoccurred within 2 months and EBVL in serum increased to levels above 4 log_{10} (peak 85 564/copies/ml = 4.93 log_{10}) in November 2001.

Due to a fulminant progression with ascites, multiple intra-abdominal lymphomas and several large lesions (diameter up to 7 cm) in the liver, and because the patient did not qualify for adoptive transfer of cytotoxic T cells or other experimental therapy, chemotherapy with standard CHOP (cyclophosphamide, adriamycin, vincristine, prednisone) was initiated in April 2002. After eight cycles of chemotherapy including cycles of anti-CD20 therapy (4×600 mg intravenously), a partial remission was
achieved with only some small liver lesions (<1 cm) remaining. However, an early relapse occurred only 2 months after the last cycle of CHOP, and salvage chemotherapy with the DHAP regimen (dexamethasone, high-dose ara C, cisplatin) was started. In addition, intravenous acyclovir was added with a significant decrease of EBVL (~2.25 log\(_{10}\)). Despite this combined therapy the disease progressed after a remission of only 4 weeks duration. The patient died of a neutropenia-associated sepsis in February 2003. Her relatives denied a necropsy.

**Discussion**

This case demonstrates a close correlation of EBVL with the course of EBV-associated AITL. In addition, we observed an impressing clinical and virological response to antiviral treatment in the early stage of this EBV-associated lymphoma but not in subsequent stages. The serum EBVL paralleled the degree of lymphadenopathy. Although AITL has been associated with EBV [5], correlations of EBVL with disease activity have been reported only rarely for AITL [17].

Correlation of EBVL with disease progression is well established in other EBV-associated cell proliferations including post-transplant lymphoproliferative disease (PTLD) [6,18], non-Hodgkin’s lymphoma in the acquired immunodeficiency syndrome [15,19], and occasionally in Hodgkin’s disease [15]. PTLD is known to be associated with higher blood levels of EBV, usually exceeding 10,000 DNA copies/ml [20,21], similar to levels observed in this patient with AITL. In our patient, the significance of EBV for disease occurrence and manifestation was further supported by the fact that EBV encoded RNA-1 (EBER-1) and the EBV latent membrane protein LMP-1, a known oncogene, were present in high amounts in situ in the examined lymph node [22]. To note, EBER-1 and LMP-1 expression may be found with clinically non-relevant EBV infection and does not necessarily prove a role for EBV in disease manifestations.

In immunosuppressed patients, an increase of latently infected resting B cells can occur, which then expand [23]. Similarly, an expansion and high proliferation rate of CD3, CD20 and CD30 positive immunoblasts was documented in our patient. In this woman an obvious immunodeficiency was not present, however, thyroid goitre and an earlier therapy with neo-mercazole may have affected cytotoxic T cells [24]. Due to the unfavourable and eventually fatal course of this EBV infection and AITL one has to assume that some sort of immune incompetence [25] or at least a critical virus–host interaction existed, leading to a damaging EBV persistence. Although we could demonstrate the presence of cytotoxic T cells against EBV in our patient, the frequency of 16 EBV cytotoxic T cell precursors per million PBMCs was low [14] as compared to healthy EBV carriers, where the frequency was found to be between 60–600/million PBMCs [26]. Also, it may well have been that reactivity against certain EBV epitopes was lacking and the strength of response was not sufficient to contain the infection.

The highly remarkable clinical and virological response to valacyclovir treatment in a patient with EBV-associated lymphoma is unprecedented. Valacyclovir resulted in a drastic, more than 3 log\(_{10}\) decrease of EBVL and a near complete disappearance of enlarged lymph nodes. Unfortunately, due to severe side effects valacyclovir could not be given for prolonged times and when antiviral therapy was given later it did not have the same clinical effect despite EBVL decrease of more than 2 log\(_{10}\). Attempts to treat EBV lymphoproliferative disorders with antiviral agents, particularly inhibitors of the thymidine kinase such as ganciclovir or acyclovir, have been undertaken in numerous contexts [27,28]. It is difficult to measure antiviral efficacy against EBV. *In vitro* assays are not ideal. However, it is well recognized that inhibition of the viral thymidine kinase can abrogate lytic infection [29]. Acyclovir, the active metabolite of the prodrug valacyclovir, is effective in this context. The thymidine kinase is usually not expressed in B cells during EBV lymphoproliferative disorders and hence there would be no rationale for treatment of EBV lymphoproliferative disorders with thymidine kinase inhibitors. However, lytic infection may be activated during stages of rapidly proliferating B-cell lymphomas as indicated by the clinical course of our case justifying the use of antivirals. Babcock and colleagues characterized the state of EBV-infected B cells from asymptomatic immunosuppressed EBV-seropositive organ transplant recipients with elevated viral loads shortly after transplantation [23]. Interestingly, the authors concluded from their study that a proportion of infected cells in the peripheral blood might have been in the lytic phase. Thus, antiviral therapy with acyclovir or ganciclovir might be expected to have an effect in the subset of infected B cells expressing lytic replication. Also, a newer study demonstrated that induction of the EBV thymidine kinase gene in latently infected EBV-positive tumour cells by arginine butyrate may lead to successful antiviral treatment with ganciclovir [29].

Most cases of AITL display a clonal expansion of T cells, a proportion of cases also clonal expansion of B cells, and occasionally diffuse large B-cell lymphomas develop. In some cases, these EBV-positive cell clones may develop into B-cell lymphomas [30]. In our patient we documented clonal expansion of B cells followed by increased appearance of immunoblasts in the early
stage of disease before treatment with valacyclovir was commenced. No specimens to further document the B-cellular clonal evolution could be obtained at later stages when responsiveness to valacyclovir declined. Although clonality in the T-cell population is common, it is not always seen as in our case. Nevertheless, cases such as ours fit within the definition and clinicopathological spectrum of AITL. We have to hypothesize that our patient experienced a proliferation of EBV-harbouring B cells similar to that also known to occur in PTLD, that is, progression from polyclonal to oligo-clonal and eventually to monoclonal disease. This analogy to PTLD, that is, the fact that in the early stage polyclonality seems to better respond to therapy than later stages displaying oligo-clonality or monoclonality [6] could explain the observed vanishing response to antiviral treatment. In our patients this progression was possibly accompanied by loss of lytic infection.

Since antiviral therapy was incapable of achieving long-term control, chemotherapy including anti-CD20-antibody therapy eventually had to be administered. Cytotoxic drug therapy is an established option in patients with AITL, and complete response rates of 30–70% have been reported in different small series [3]. However, virtually all patients relapse, and despite some promising reports on the role of high dose chemotherapy with autologous bone marrow support [31], AITL seems to be one of the high grade lymphomas with the worst prognosis.

In conclusion, this case describes an EBV-associated lymphoma and demonstrates that antiviral treatment with valacyclovir exhibited a remarkable regression at an early stage. Although in vitro studies do not support valacyclovir activity against EBV, this compound ought to be further investigated in the treatment of EBV-associated AITL, PTLD or other EBV-associated lymphomagenesis, such as, in combination with anti-neoplastic drugs.

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References


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