Case Report
A probable identical Epstein-Barr virus clone-positive composite lymphoma with aggressive natural killer-cell leukemia and cytotoxic T-cell lymphoma

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Abstract: The patient was a 52-year old woman with a history of mosquito-bite hypersensitivity since childhood. In July 2011, she developed pyrexia, headaches, and nausea, and Epstein-Barr virus (EBV)-positive aggressive natural killer leukemia (ANKL) was diagnosed on the basis of both a peripheral blood and bone marrow examination. An inguinal lymph node biopsy, on the other hand, revealed EBV-positive cytotoxic T-cell lymphoma plus the presence of a small number of EBV-positive ANKL cells, and a diagnosis of EBV-positive composite lymphoma was made. Both the cytotoxic T-cell lymphoma and ANKL exhibited EBV terminal repeat (Southern blot analysis) monoclonal patterns, and they were almost the same size, approximately 9.0 kb. If it was the identical EBV clone, it is possible that EBV infected progenitor cells common to both NK cells and T cells, that the progenitor cells then differentiated into NK cells and T cells, a chronic active Epstein-Barr virus infection developed, and neoplastic transformation occurred. If it was not the identical EBV clone, fairly similar EBVs must have infected NK cells and T cells separately, and they then underwent neoplastic transformation. Because the mechanism by which EBV infects NK cells or T cells is still unknown, we concluded that this case is also important from the standpoint of elucidating it. We are currently in the process of conducting gene analyses to determine whether the fairly similar EBVs that infected the ANKL and cytotoxic T-cell lymphoma are the identical clone.

Keywords: Aggressive NK leukemia, Epstein-Barr virus, cytotoxic T-cell lymphoma, composite lymphoma, large granular lymphocytes

Introduction
The mechanism of infection in Epstein-Barr virus (EBV)-associated T/NK-cell lymphoproliferative disease is still unknown, but it is a systemic disease in which EBV-infected T/NK cells proliferate clonally [1, 2]. One of its forms, aggressive natural killer leukemia (ANKL), is a disease in which NK cells having the morphology of large granular lymphocytes (LGLs), which are regarded as the mature form, and it is a tumor that takes the form of leukemia [3]. ANKL is mostly observed teenagers and young adults in Asia [4]. Many cases of ANKL are complicated by hemophagocytic syndrome, disseminated intravascular coagulation, or multiple organ failure, and it has a very poor prognosis, with a median survival time that is less than 2 months [5]. A clonal EBV terminal repeat is detected in the tumor cells in almost all of the cases [6]. During the course of the disease approximately 12% of the cases of EBV-associated T/NK cell lymphoproliferative disease progress to overt lymphoma/leukemia, including ANKL, and during its course a new chromosomal aberration occurs in approximately 7% of the cases [7]. Thus, it appears that a mutation of a tumor sup-
EBV positive composite lymphoma

Figure 1. Cytologic findings of the peripheral blood (A, x 1000) and biopsy findings of the bone marrow (B-D, x 600) and lymph node (E-N, x 600). A: Giemsa, large granular lymphocytes with clear nucleoli; B: Hematoxylin and eosin (HE), aggressive natural killer leukemia (ANKL) cells infiltration; C: EBER-positive; D: CD56-positive; E: HE, loss of architecture of the lymph node, and diffuse tumor cell infiltration; F: Double-staining for EBER (brown) and CD3 (blue), positive for both; G: Double staining for EBER (brown) and CD5 (blue), CD5-negative ANKL and CD5-positive cytotoxic T-cell lymphoma; H: Double-staining for EBER (brown) and CD20 (blue), EBER-positive cells were all CD20-negative; I: CD4-negative; J: CD8-positive; K: Highly Ki-67-positive; L: TIA-1-positive; M: Granzyme B-positive; N: CD56-negative.
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Figure 2. Two-color flow-cytometric analysis of mononuclear cells in the peripheral blood. Surface phenotype examination of the EBV-infected cells showed that the cells were negative for CD3, CD4, CD5, CD8, CD19, TCRαβ and TCRγδ, and strongly positive for CD56 (yellow square); some of the cells also showed weakly positive staining for CD16.

Compressor gene/oncogene occurs in the EBV-infected cells that have proliferated clonally, and it gradually progresses to ANKL, etc [7]. EBV clones are often detected in both NK cells and T cells in EBV-associated T/NK cell lymphoproliferative disease [8-10]. However, since there have been no reports of cases like our own in which EBV infected the same lymph node (LN), and the cells underwent neoplastic transformation, we concluded that this is a very valuable case.

Case report

The patient is a 52-year-old woman with a history of mosquito-bite hypersensitivity since childhood who began to develop nausea, anorexia, pyrexia around 40.0°C, and headaches in July 2011, and was examined at another hospital. Splenomegaly, hepatobiliary enzyme elevations, thrombocytopenia (5.0 x 10^4/μL), and a white blood cells (WBC) count of 6700/μL (77% lymphocytes) were found, and the patient was admitted to that hospital, but she spontaneously recovered and was discharged in September. The patient was examined in our hospital in October 2011. Her WBC count was 7100/μL, and 65% of them were LGLs that had a clear nucleolus (Figure 1A). The serum lactate dehydrogenase (LDH: 341 IU/L) and serum soluble interleukin-2 receptor (sIL-2R: 1250 U/mL) values were mildly elevat-
ed. Numerous LGLs containing clear nucleoli were also seen in the bone marrow (BM). An abdominal computed tomography examination revealed hepatosplenomegaly (data not shown).

In November 2011, generalized fatigue, pyrexia, and a consciousness disorder were noted, and the patient was admitted to our hospital. Examination on admission revealed a body temperature of 38.5°C, slight drowsiness, pal-

Figure 3. Monoclonal Epstein-Barr virus terminal repeat (A-C) and T cell receptor rearrangement (D) by southern blot analysis. Monoclonal Epstein-Barr virus (EBV) terminal repeat (A-C); (A) Peripheral blood (PB); (B) Bone marrow (BM); (C) Lymph node (LN); (A-C) Monoclonal bands were detected (black arrow); lane M, size markers (λDNA/HindIII); lane 1, positive control; lane 2, negative control; lane 3, patient, restriction enzyme (RE) BamHI; T cell receptor Cβ1 rearrangement (D); (D) LN; the CTβ DNA probe detected rearrangement bands (red arrow); lane 1, RE BamHI; lane 2, RE EcoR V; lane 3, RE Hind III.
pation of the spleen 4 fingerbreadths below the costal margin, and 3 LNs measuring 1.1-1.5 cm were palpated in the inguinal region bilaterally. There were no abnormal neurological findings. Laboratory data included the following: WBC 5100/μL (61% LGLs with clear nucleoli), Hemoglobin (Hb) 11.7 g/dL, platelet (Plt) 5.3 x 10^9/L, aspartate aminotransferase (AST) 258 IU/L, alanine aminotransferase (ALT) 204 IU/L, serum LDH 861 IU/L, serum ferritin 666 ng/mL, serum sIL-2R 4700 U/mL, which was elevated. The anti-EBV capsid antigen IgG titer was 1:320, the anti-EBV early antigen IgG titer was 1:160, and the anti-EBV nuclear antigen IgG titer was 1:20. The Peripheral mononuclear EBV load was 2.4 x 10^6 copies/μgDNA, The plasma EBV DNA load was 1.1 x 10^5 copies/mL. \(^{18}\)F-fluoro-2-deoxy-D-glucose (FDG)-position emission tomography/computed tomography revealed mild FDG accumulation in the right cervical, right axillary, right external iliac, and bilateral inguinal LNs (maximum standardized uptake value [SUVmax] 2.9), and hepatosplenomegaly was also observed.

Flow cytometry of peripheral blood (PB) mononuclear cells showed that 55% were EBV-encoded small RNA in situ hybridization (EBERISH)-positive and double staining was performed with each surface marker (Figure 2) [11]. The surface phenotype of the EBV-infected cells showed that CD3, CD4, CD5, CD8, T cell receptor (TCR) αβ, and TCRγδ were all negative, and CD56 was strongly positive, and some of them were weakly positive for CD16.

The patient’s PB, BM, and inguinal LN yielded monoclonal bands in which the EBV terminal repeats in the vicinity of 9 kb were fairly similar (Figure 3A-C). Analysis of TCRCβ1 revealed a rearrangement band (Figure 3D). However, no rearrangements of TCRCβ1, TCRγ, or immunoglobulin heavy chain joining region (IgHJH) were observed in the PB or BM (data not shown).

LGLs infiltration was observed in approximately 40% in the BM, and increase in macrophages and prominent hemophagocytosis were seen. FCM of the patient’s BM showed infiltration by ANKL cells, the same as in the PB (data not shown). High-power microscopic examination (x 600) of a BM biopsy specimen also showed EBER-positive, CD56-positive ANKL tumor cell infiltration (Figure 1B-D). Based on the above findings, ANKL positive for EBV that appeared to be the identical clone was observed in the patient’s PB and BM.

Next, we will describe the pathological findings in the inguinal LN at the x 600 magnification (Figure 1E-N). HE staining showed that the basic LN architecture had been lost, and diffuse tumor cell infiltration was observed (Figure 1E). The diagnosis of this tumor appeared to be an approximately 90% or more EBV-positive, CD8-positive cytotoxic T-cell lymphoma, because it was EBER-positive, CD3-negative, CD4-negative or weakly positive, CD5-negative, CD8-positive, CD20-negative, highly Ki-67-positive, TIA-1 and Granzyme B-positive, and CD56-negative (Figure 1F-N). However, infiltration by CD56-positive ANKL cells to a level of several percentage points was also observed (Figure 1N). Under high power, we observed a pleomorphic tumor, and double-staining for EBER and CD3 (Figure 1F) showed that it was positive for both. On the other hand, double staining for EBER and CD5 (Figure 1G) revealed the presence of both an EBER-positive, CD5-negative group and an EBER-positive, CD5-positive group. Both the CD5-negative ANKL and the CD5-positive cytotoxic T-cell lymphoma appeared to be EBV-positive. The EBER-positive cells were all CD20-negative, and EBV infection of B cells was ruled out. The double staining for EBER and CD56, however, was not very successful technically.

In addition, there was a TCRCβ1 rearrangement in the inguinal LN, and it was diagnosed as a T-cell tumor (Figure 3D). We also observed a monoclonal band of the EBV terminal repeat in the vicinity of 9 kb in the inguinal LN that was almost exactly the same as in the PB and BM (Figure 3C). The EBER-positive cells in the LN were positive in both the cytotoxic T-cell lymphoma and ANKL cells, suggesting that the EBV might be the identical clone.

During the clinical course after the patient was admitted she was treated with a steroid, but the hemophagocytic syndrome rapidly progressed, and the patient died on the 8th day after admission. The autopsy findings showed that CD5-negative, EBER-positive ANKL cells had infiltrated the LNs, liver, and spleen (data not shown).

Discussion

A mere two cases of ANKL complicating another lymphoma have ever been reported. In the first case the patient was a 74-year-old woman in whom a complete response was achieved with CHVmp chemotherapy (cyclophospha-
NK cells and T cells are generally thought to differentiate from the same precursor cells [14]. In present case, both the ANKL and cytotoxic T-cell lymphoma exhibited EBV terminal repeat monoclonal patterns, and they were almost the same size, approximately 9.0 kb. If EBV had infected these common precursor cells, the EBV genome of the ANKL and cytotoxic T-cell lymphoma might be identical. Since ANKL would have a growth advantage in such a situation, progression of the ANKL would be more prominent. A chronic active EBV (CAEBV) infection developed, and neoplastic transformation occurred. If EBV had infected the two tumors separately, usually the EBVs would be different. In view of the current state of affairs, in which the mode of EBV infection of NK and T cells is unknown, since it is possible to demonstrate the stage of differentiation at the time when EBV infected the cells, we concluded that analyzing the EBV genome of the two tumors, i.e., the ANKL and cytotoxic T-cell lymphoma to determine whether they are the same or different, and we plan to publish the results in the future. EBV is known to infect B cells via CD21 [15]. However, even now the receptors that mediate EBV infection of T cells or NK cells remain unknown. Analyzing whether EBV infected via the EBV receptors of the progenitor cells that are common to both T cells and NK cells or whether EBV infected via separate EBV receptors of the T cells and NK cells is very important to research on the mode of infection of CAEBV, and our case can be described as very valuable.

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Disclosure of conflict of interest

None.

References

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