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Case Report

Severe mosquito bite allergy with clonally proliferating EBV-positive T-cells developing into peripheral T-cell lymphoma: a case report

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Abstract: A 28-year-old woman suffering from a severe mosquito bite allergy (sMBA) since her childhood for more than 20 years, was referred to our hospital with fever, liver dysfunction, and pancytopenia. Clonally proliferating CD4- and CD8-double positive and Epstein-Barr virus (EBV) positive T cells were detected in the peripheral blood (PB). She was diagnosed with EBV-positive lymphoproliferative disease. Two months after the admission, a tumor developed in her left lung. A biopsy was performed and the infiltration of atypical EBV-positive cells whose phenotype was identical to that in the PB was detected in the lesion. The diagnosis of EBV-positive Peripheral T-cell lymphoma, not otherwise specified (PCTL-NOS) was made following this. The lymphoma was resistant to chemotherapy and the patient passed away from sudden ventricular tachycardia followed by cardiac arrest. An autopsy revealed infiltration of lymphoma cells into the heart, lung, liver, spleen, and intestine. This is the first reported case that EBV-positive T-cells detected during the period of sMBA were confirmed to develop into PCTL-NOS. PCTL-NOS in this case was resistant to the given treatment and the poor outcome supports the notion that therapeutic intervention in the early phase of sMBA before transformation, may be crucially needed.

Keywords: EBV-positive lymphoproliferative diseases, severe mosquito bite allergy, hemophagocytic lymphohistiocytosis, peripheral T-cell lymphoma, not otherwise specified

Introduction

Severe mosquito bite allergy (sMBA) is a rare disease characterized by local skin inflammation and systemic symptoms and signs such as high fever, lymphadenopathy and hepatopathy following the bites of *Aedes (Stegomyia) albopictus* also known as the Asian tiger mosquito. The puncture sites ulcerate and although they can be cured within a month, they often leave scars. sMBA occurs due to the hyper-reactive response of the patients' lymphocytes to the mosquito's saliva [1]. In 1997, Ishihara et al. detected a monoclonal proliferation of Epstein-Barr virus (EBV)-positive T and natural killer (NK) cells in the peripheral blood of sMBA patients [2]. The following reports indicated that sMBA could lead to the development of fatal disorders such as T- or NK-cell lymphoma or hemophagocytic lymphohistiocytosis (HLH). Therefore, sMBA is now classified as a T- or NK-cell neoplasm in the WHO classification of EBV-positive T/NK lymphoproliferative diseases (EBV-T/NK-LPDs) [3]. There are two other EBV-T/NK-LPDs: chronic active EBV infection and hydroa vacciniforme. These diseases also involve EBV-positive clonally proliferating T- or NK-cells in the peripheral blood and have similar clinical courses as sMBA. As these conditions are rare, there have been a small number of case reports. Additionally, there have been no detailed reports of sMBA with EBV-positive T-cells followed by the development of peripheral T-cell lymphoma, not otherwise specified (PCTL-NOS). Here, we report a case of a patient with CD4- and CD8-double-positive T-cell-type sMBA developing PCTL-NOS and discuss the clinical features and management.

Case report

A 28-year-old woman suffering from sMBA for more than 20 years was referred to our hospital with fever, liver dysfunction, and pancytopenia.

Table 1. Laboratory data on admission

Peripheral blood		Biochemistry		Anti-EBV antibodies	
WBC	2100/μl	TP	6.4 g/dl	anti-VCA-IgG	×10240
Myelo	1%	BUN	9 mg/dl	anti-VCA-IgM	< 10
Stab	5%	Alb	1.8 g/dl	anti-EA-DRIgG	×1280
Seg	68%	Cre	0.39 mg	anti-VCA-IgA	< 10
Lym	20%	LD	1052 IU/l	anti-EBNA	×40
Mo	5%	AST	162 IU/l		
Aty. Lym	1%	ALT	78 IU/l	EBV-DNA load of each lymphocyte fraction in PB	
RBC	235×10^4/μl	γ-GTP	93 IU/l	Whole blood	2.3×10^5 copies/μg DNA
Hb	6.7 g/dl	ALP	993 IU/l	PBMC	4.9×10^4 copies/μg DNA
Ht	20.0%	T-Bil	7.7 mg/dl	CD19	N.D.
Plt	5.3×10^4/μl	D-Bil	5.8 mg/dl	CD4	3.0×10^5 copies/μg DNA
Ret	45.9‰	T-Chol	61 mg/dl	CD8	7.6×10^4 copies/μg DNA
		TG	331 mg/dl	CD56	N.D.
		CK	11 IU/l	Others	N.D.
Coagulation		Glu	122 mg/dl	Serum	1.2×10^4 copies/μg DNA
		Ferritin	1978.0 ng/dl		
PT-INR	1.41	CRP	3.15 mg/dl		
APTT	60.1 sec	IgG	3080 mg/dl		
Fibrinogen	74 mg/dl	IgM	154 mg/dl		
FDP	4.8 μg/ml	IgA	430 mg/dl		
ATIII	41.6%	sIL-2R	2047 U/ml		

VCA; viral capsid antigen; PBMC; peripheral blood mononuclear cell; EBNA; Epstein-Barr virus nuclear antigen; N. D.; not detected.

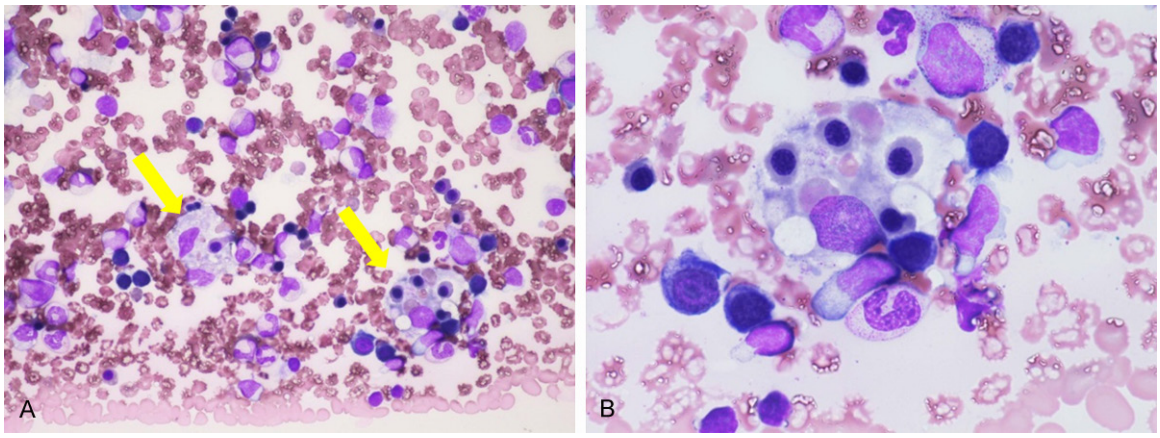

Figure 1. Bone marrow aspiration at admission. May-Giemsa staining. A: Hemophagocytic cells were detected (yellow arrows). B: A macrophage with engulfed erythrocytes and granulocytes.

She had suffered from severe inflammation of the skin and high fever following mosquito bites since her childhood. In November 201X, more than 20 years after the onset of sMBA, she was admitted to a community hospital with gastrointestinal hemorrhaging. She had high fever, splenomegaly, pancytopenia, coagulation abnormality and increased levels of transaminases. Elevation of anti-EBV viral capsid antigen (VCA) antibodies, increased EBV-DNA load and ferritin in the peripheral blood were determined. EBV-associated disease was suspected and she was referred to our hospital at the beginning of December.

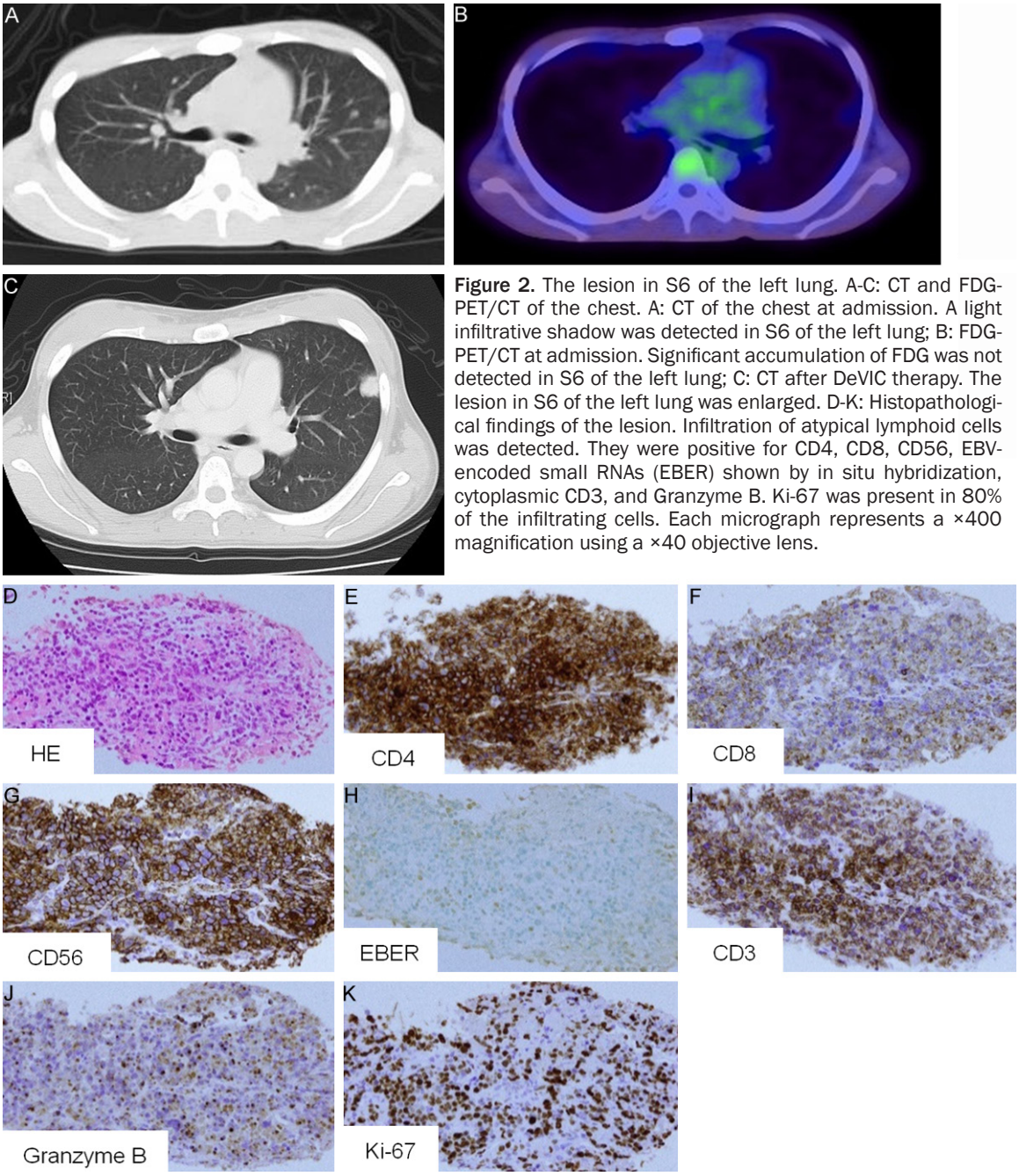

Figure 2. The lesion in S6 of the left lung. A-C: CT and FDG-PET/CT of the chest. A: CT of the chest at admission. A light infiltrative shadow was detected in S6 of the left lung; B: FDG-PET/CT at admission. Significant accumulation of FDG was not detected in S6 of the left lung; C: CT after DeVIC therapy. The lesion in S6 of the left lung was enlarged. D-K: Histopathological findings of the lesion. Infiltration of atypical lymphoid cells was detected. They were positive for CD4, CD8, CD56, EBV-encoded small RNAs (EBER) shown by in situ hybridization, cytoplasmic CD3, and Granzyme B. Ki-67 was present in 80% of the infiltrating cells. Each micrograph represents a ×400 magnification using a ×40 objective lens.

Laboratory findings on admission are presented in **Table 1**. Pancytopenia, disseminated intravascular coagulopathy, liver dysfunction and elevated serum ferritin and triglyceride levels were detected. When anti-EBV antibodies were tested, there was a high titer of the anti-VCA-IgG antibody and negativity for anti-VCA-IgM and anti-Epstein-Barr virus nuclear antigen antibodies (**Table 1**). From these findings, a diagnosis of primary EBV infection was excluded. Bone marrow aspiration was performed which showed hemophagocytic macrophages (**Figure 1**). However, no atypical lymphocytes were detected. Whole body positron emission tomography/computed tomography with 2-deoxy-2-[^{18}F] fluoro-D-glucose (FDG-PET/CT) showed hepatosplenomegaly. A consolidation was determined in S6 of the left lung, but no significant accumulation of FDG (**Figure 2A**, **2B**) was observed. EBV-DNA load in the peripheral

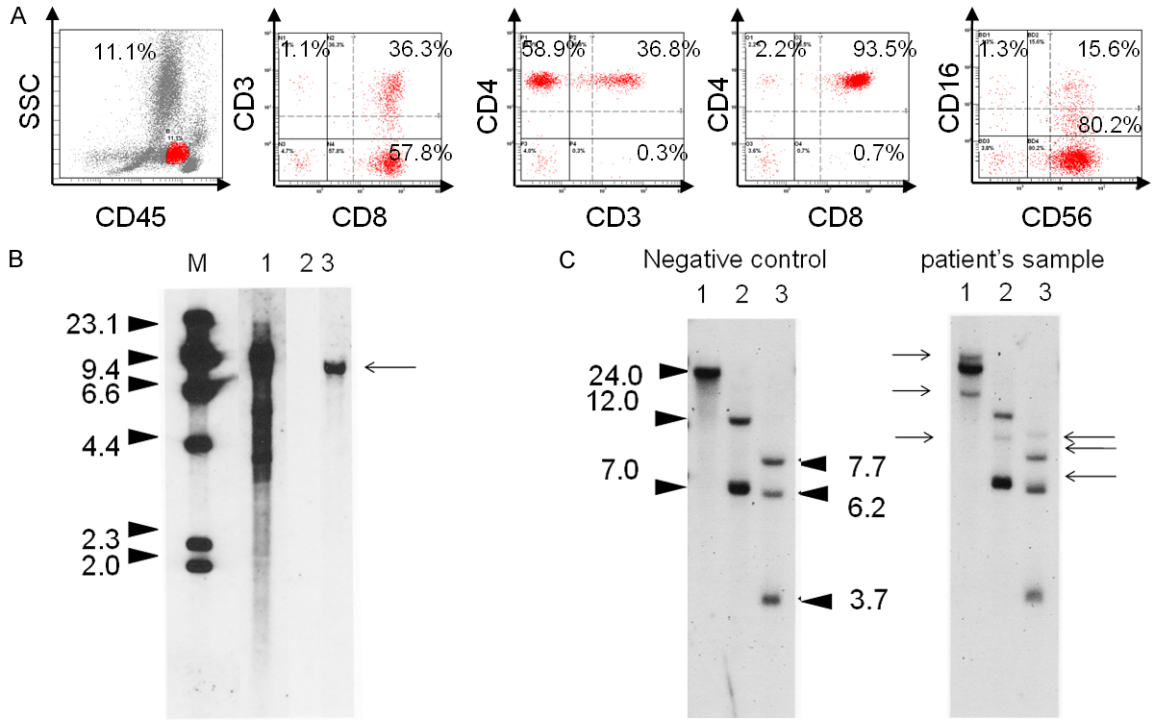

Figure 3. Analysis of the peripheral blood mononuclear cells from the present case at admission. A: Flow cytometry. Most cells were CD4-, 8-, and 56-positive. Among them, CD3-negative cells were present. B: Southern blot analysis for EBV-terminal repeats. A monoclonal band (arrow) was detected in the patient's sample. M: molecular marker. 1: positive control. 2: negative control. 3: the patient's sample. C: Southern blot analysis for T cell receptor gene rearrangement of Cβ1, 2. Monoclonal bands (arrows) were detected in the patient's samples. 1: Bam HI, 2: Eco RV, 3: Hind III.

blood increased to 2.3×10^5 copies/μg DNA. Flow cytometry of the peripheral blood mononuclear cells (PBMCs) revealed CD4- and CD8-double-positive cells. They also expressed positivity for CD56, and some were negative for surface CD3 (**Figure 3A**). Southern blot analysis showed a monoclonal band of EBV terminal repeats in PBMCs (**Figure 3B**). Analysis for T-cell receptor Cβ1 rearrangement also showed clonal bands, indicating that monoclonal proliferation of EBV-infected T-cells had occurred (**Figure 3C**). Detection and isolation of EBV-infected cells were performed as described previously [4]. Briefly, PBMCs were isolated and separated into CD4-, CD8-, and CD56-positive fractions using antibody-conjugated magnetic beads (IMag Human CD4, CD8, and CD56 Particles-DM; BD Biosciences, Sparks, MD, USA) according to the manufacturer's instructions. The EBV-DNA load in each fraction was then measured via real-time reverse transcriptase (RT)-polymerase chain reaction (PCR) [5] using a TaqMan system (Applied Biosystems, Foster City, CA, USA). The fraction with the highest titer was assumed to contain EBV-positive cells. We detected EBV infection in CD4- and CD8-positive cells (**Table 1**). From these results, we diagnosed HLH developed from T-cell-type sMBA, of which the infected cells were CD4- and CD8-double-positive.

We decided that the patient should receive hematopoietic stem cell transplantation (HSCT) and started searching for a donor. Simultaneously, we performed cooling therapy (prednisolone 1 mg/kg/day, cyclosporine 3 mg/kg/day, and etoposide 150 mg/m^2/week) [6], but the HLH became resistant to this treatment within a month. To increase the intensity of the treatment, we started DeVIC therapy (ifosfamide 1500 mg/m^2/day on days 1-3, dexamethasone 40 mg/day on days 1-3, etoposide 100 mg/m^2/day on days 1-3, and carboplatin 300 mg/m^2/day on day 1). However, during the second course of DeVIC therapy, contrast-enhanced CT of the chest showed enlargement of the consolidation in the left lung (**Figure 2C**). A CT-guided lung biopsy detected CD4-, 8-, 56-, and EBV-positive atypical lymphocytes (**Figure**

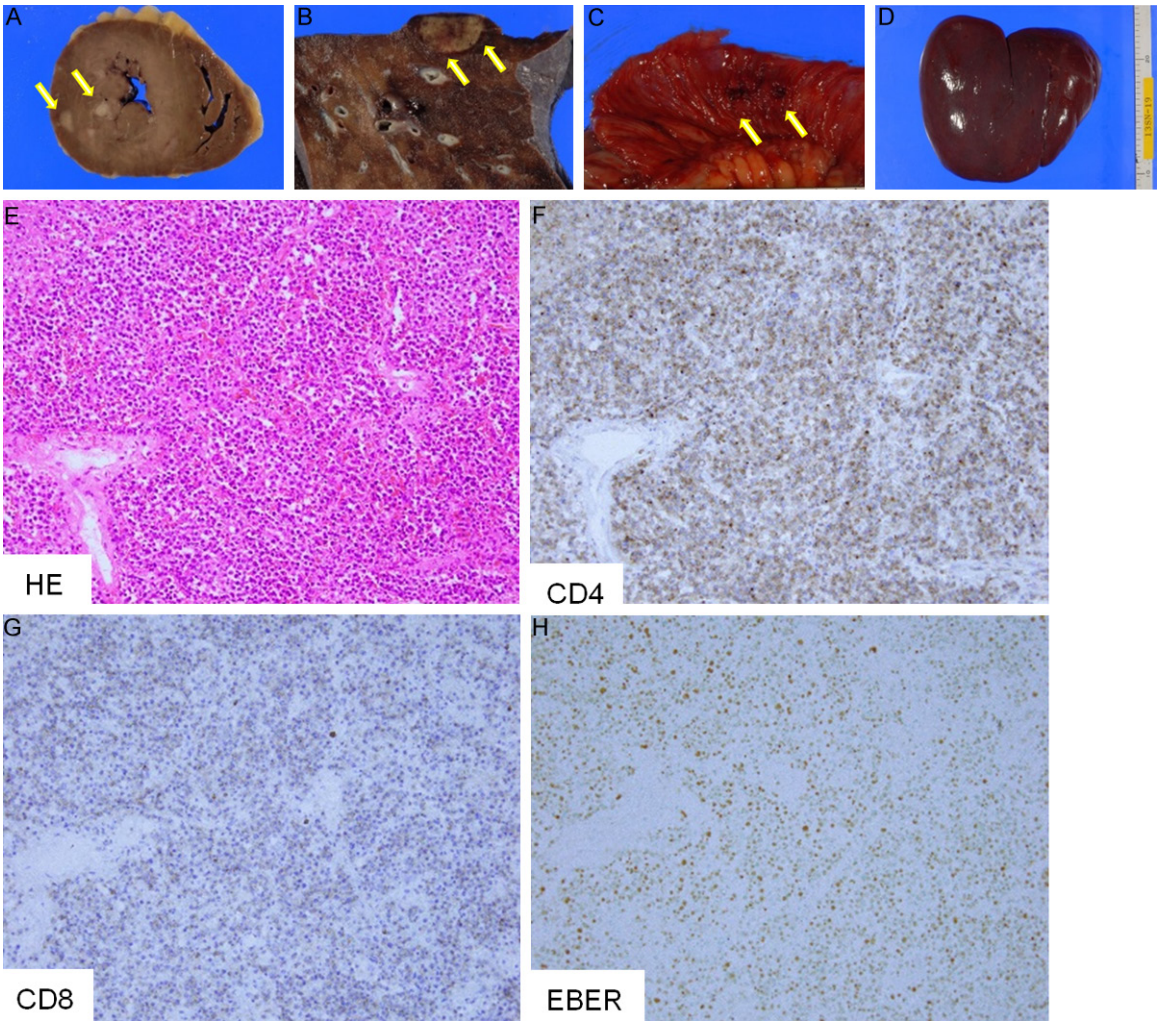

Figure 4. The autopsy specimen. (A-D) Macroscopic findings of the organs with infiltration of tumor cells. The yellow arrows indicate lesions of the heart (A), the left lung (B), and the small intestine (C). Splenomegaly was detected (D). (E-H) Histopathological findings of the lesion in the left lung. Massive infiltration of CD4-, CD8-, and EBV-positive cells was detected.

2D-H), with identical phenotypes to those in the peripheral blood (**Figure 3A**). These cells were also positive for cytoplasmic CD3, Granzyme B, and Ki-67. Ki-67 was present in 80% of the infiltrating cells (**Figure 2I-K**). From these results, we diagnosed PCTL-NOS developed from T-cell-type sMBA. We added ESHAP therapy (etoposide 40 mg/m^2/day on days 1-4, methylprednisolone 500 mg/day on days 1-5, cisplatin 25 mg/m^2/day on days 1-4, and cytarabine 2000 mg/m^2/day on day 5) for PCTL-NOS. However, the tumor became enlarged and the HLH recurred. In April 201X, sudden ventricular tachycardia followed by cardiac arrest occurred. The patient did not respond to resuscitation and passed away. The clinical course lasted 20 years from the onset of sMBA, 4 months from the onset of HLH, and 1 month from the onset of PCTL-NOS.

An autopsy revealed multiple tumors in the myocardium, the S6 of the left lung and the intestine (**Figure 4A-C**). Massive splenomegaly was detected (**Figure 4D**). Nodal or diffuse infiltration of lymphocytes was observed in the heart, lung, liver, spleen, and intestine, and their phenotypes were identical to those of the tumor cells detected in the biopsy (**Figure 4E-H**). Systemic dissemination of PCTL-NOS was determined and infiltration into the myocardium was considered to be the direct cause of the death.

Discussion

EBV-infected cells are NK-cells in 89% of sMBA cases and T-cells in the remaining 11% of cases [7]. The majority of sMBA stems from NK-cell infection and accordingly, the most reported lymphomas developing from sMBA are the NK-cell lymphomas. To our knowledge, only two cases of T-cell lymphoma from sMBA have been reported [7, 8]. The present report is the first case of sMBA to develop into PCTL-NOS in which the lymphocytes had an identical phenotype to that of the EBV-infected clonally proliferating T-cells detected during the sMBA period. The PCTL-NOS was thought to develop from the infected cells. Nakamura et al. reported that the expression of the activation-induced cytidine deaminase, which induces gene mutation, was increased in EBV-positive T- or NK-cells in chronic active EBV infection [9]. These reports indicated that there are mechanisms to promote survival as well as gene mutation in EBV-infected T- and NK-cells. In this case, PCTL-NOS showed rapid progression, extranodal infiltration, and resistance to conventional chemotherapies. Clarification of how transformation occurs and establishment of effective chemotherapy is very much needed.

Miyake et al. reported a retrospective analysis of nine cases of sMBA. The report indicated that the cumulative survival rates were below 50% at 4 years [10]. Kimura et al. performed a prospective analysis of 108 cases of EBV-T/NK-LPDs and reported that 4 of 9 patients with sMBA passed away because of HLH or lymphoid neoplasms [7]. These results indicate that the prognosis of sMBA is poor. However, the appropriate time to start treatment for sMBA has not been determined as the disease is asymptomatic without mosquito bites. Recently, we reported a retrospective analysis of EBV-T/NK-LPDs treated with HSCT that included two cases of sMBA [11]. Among them, one patient who received an HSCT with inactive disease survived with no reported symptoms with consequent disappearance of EBV-DNA in their peripheral blood. In contrast, the patient who developed HLH, was resistant to chemotherapy. The patient received HSCT on development of HLH and passed away soon after the transplantation. Our results indicate that the prognosis of the patients who received an HSCT for EBV-T/NK-LPD, which had already developed into HLH, was significantly poorer than those who had not. Therapeutic intervention in the early phase of sMBA, before transformation, may be crucial.

Disclosure of conflict of interest

None.

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