

Development of Extranodal NK/T-cell Lymphoma Nasal Type in Cerebrum Following Epstein-Barr Virus-positive Uveitis

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Abstract

A 74-year-old woman developed bilateral uveitis with high Epstein-Barr virus (EBV) DNA load in the vitreous fluid without lymphoma cells. Four years after the onset, T2-weighted contrast-enhanced MRI revealed hyperintense lesions in the right occipital and parietal lobe. A biopsy resulted in the diagnosis of extranodal NK/T-cell lymphoma nasal type (ENKL). The repeat region of *LMP1*, an EBV gene, detected in the brain lesion was identical to that detected in the vitreous fluid. ENKL of the central nervous system is quite rare, and the pathogenesis has not been determined. The lymphoma in this case might have been closely associated with the EBV-positive uveitis.

Key words: extranodal NK/T-cell lymphoma nasal type, central nervous system, uveitis, Epstein-Barr virus

(Intern Med 56: 1409-1414, 2017)

(DOI: 10.2169/internalmedicine.56.7573)

Introduction

Extranodal NK/T-cell lymphoma nasal type (ENKL) is a rare NK cell neoplasm with a geographical preference for East Asia with Epstein-Barr virus (EBV)-positive neoplastic cells. The most commonly involved site is the nasal cavity; however, it can additionally occur in the upper respiratory tract, skin, soft tissue, gastrointestinal tract, and testes (1). In contrast, the ocular region and central nervous system (CNS) are rarely involved as initial sites for ENKL lesions. We herein report a woman with cerebral ENKL which developed after the onset of EBV-positive uveitis.

Case Report

A 74-year-old woman was referred to our hospital due to

steroid-resistant bilateral uveitis persisting for more than 3 years. The patient had no significant medical history and was seronegative for human immunodeficiency virus (HIV). She had undergone vitrectomy of the right eye that was negative for malignant cells. DNA extracted from the vitreous fluid was negative for immunoglobulin heavy locus (IgH) rearrangements, as determined by polymerase chain reaction (PCR). The levels of interleukin (IL)-10 and IL-6 in the vitreous fluid were 51 pg/mL and 712 pg/mL, respectively. The IL-10/IL-6 ratio was <1.0, which was atypical for a diagnosis of vitreoretinal lymphoma (2).

On admission to our hospital, the visual acuity of the right eye was almost lost. The fundoscopic image of the left eye obtained on admission is shown in Fig. 1A. An analysis after diagnostic pars plana vitrectomy of the left eye did not detect any malignant cells. DNA extracted from the vitreous fluid was negative for both IgH and T-cell receptor (TCR)

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Received for publication April 13, 2016; Accepted for publication September 28, 2016

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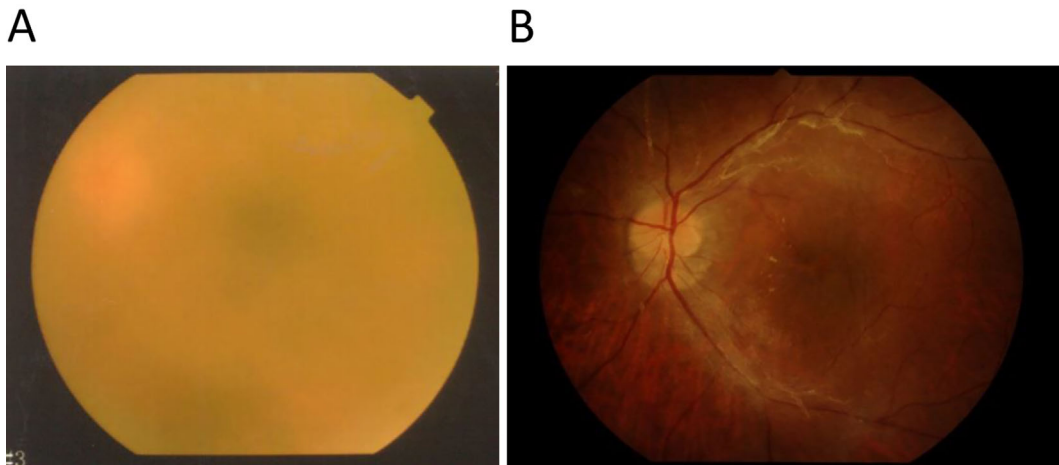


Figure 1. Color fundus images of the left eye over the course of disease. (A) An image of the fundus at the initial visit. The details of the fundus are not visible due to the dense vitreous opacity. (B) An image of the fundus one month after intravitreal methotrexate treatment administered six times, showing complete resolution of the optic and retinal edema.

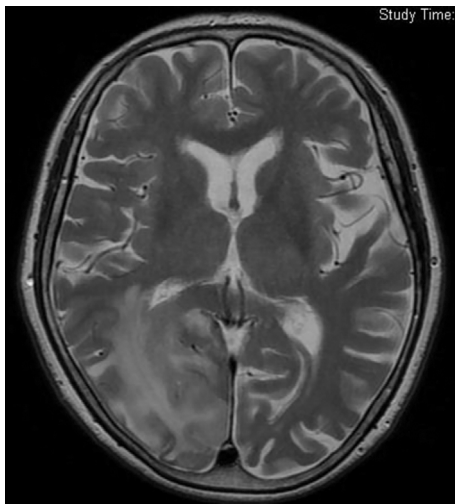


Figure 2. Magnetic resonance imaging (MRI) of the CNS lesion. MRI revealed hyperintense lesions with T2-weighted fluid-attenuated inversion recovery (FLAIR) in the right occipital lobe.

rearrangements, as determined by PCR. These findings did not support that the uveitis occurred because of lymphoma. The concentrations of IL-10 and IL-6 were 53 pg/mL and 430 pg/mL, respectively. The ratio of IL10/IL6 of the left vitreous fluid was <1.0, similar to that in the right vitreous fluid, as indicated above. Screening for infectious diseases by quantitative multiplex PCR of the vitreous fluid DNA was performed for the detection of herpes simplex virus types 1 and 2, varicella-zoster virus, EBV, cytomegalovirus, human herpes viruses 6-8, *Toxoplasma gondii*, bacterial 16S, fungal 18S/28S, and mycobacterium tuberculosis, according to our previously described method (3). The PCR results were negative for all tested pathogens, except for EBV DNA, which was detected at 5.47×10^5 copies/mL. Her anti-EBV antibody titers were 1:80 for anti-viral capsid antigen

(VCA)-IgG and 1:10 for anti-EB nuclear antigen (EBNA). Anti-VCA-IgM antibody was undetectable. An otolaryngological examination was negative for nasal lymphoma. EBV DNA was negative in the peripheral blood, and a systemic examination including the nasal cavity with 2-Deoxy-2-[^{18}F] fluoro-D-glucose positron emission tomography (PET)/CT (FDG-PET/CT) did not detect any lesions. We could not determine the diagnosis of lymphoma and injected subtenon triamcinolone acetonide into the left ocular lesion. However, this failed to improve her symptoms. In order to preserve her visual acuity, 400 μg methotrexate (MTX) weekly for 6 weeks, based on the treatment protocol for vitreoretinal lymphoma (4), was administered into the left vitreous following the approval of the Institutional Review Board. The treatment resulted in a dramatic improvement, and the patient achieved complete response (CR; Fig. 1B).

However, two years after the MTX treatment and four years after the onset of symptoms, the uveitis of the right eye rapidly progressed. Because of severe ocular pain and loss of visual acuity that was considered irreversible, the patient underwent enucleation of the right eye. A pathological examination revealed massive necrosis and the absence of lymphoma cells (data not shown). While the symptoms were resolved, the patient subsequently underwent both an ophthalmological examination and magnetic resonance imaging (MRI) for the head and neck.

Two years and two months after enucleation, multiple hyperintense lesions were detected on T2-weighted fluid-attenuated inversion recovery (FLAIR) imaging MRI in the right occipital and parietal lobes and the precentral gyrus, and a left visual field defect was detected on an ophthalmological evaluation (Fig. 2). The tumor was suspected to be high-grade glioma and was removed by craniotomy; however, the pathological diagnosis was ENKL. As shown in Fig. 3, the infiltrating cells in the pathological specimen were positive for CD3, CD56, and TIA1. The tumor cells

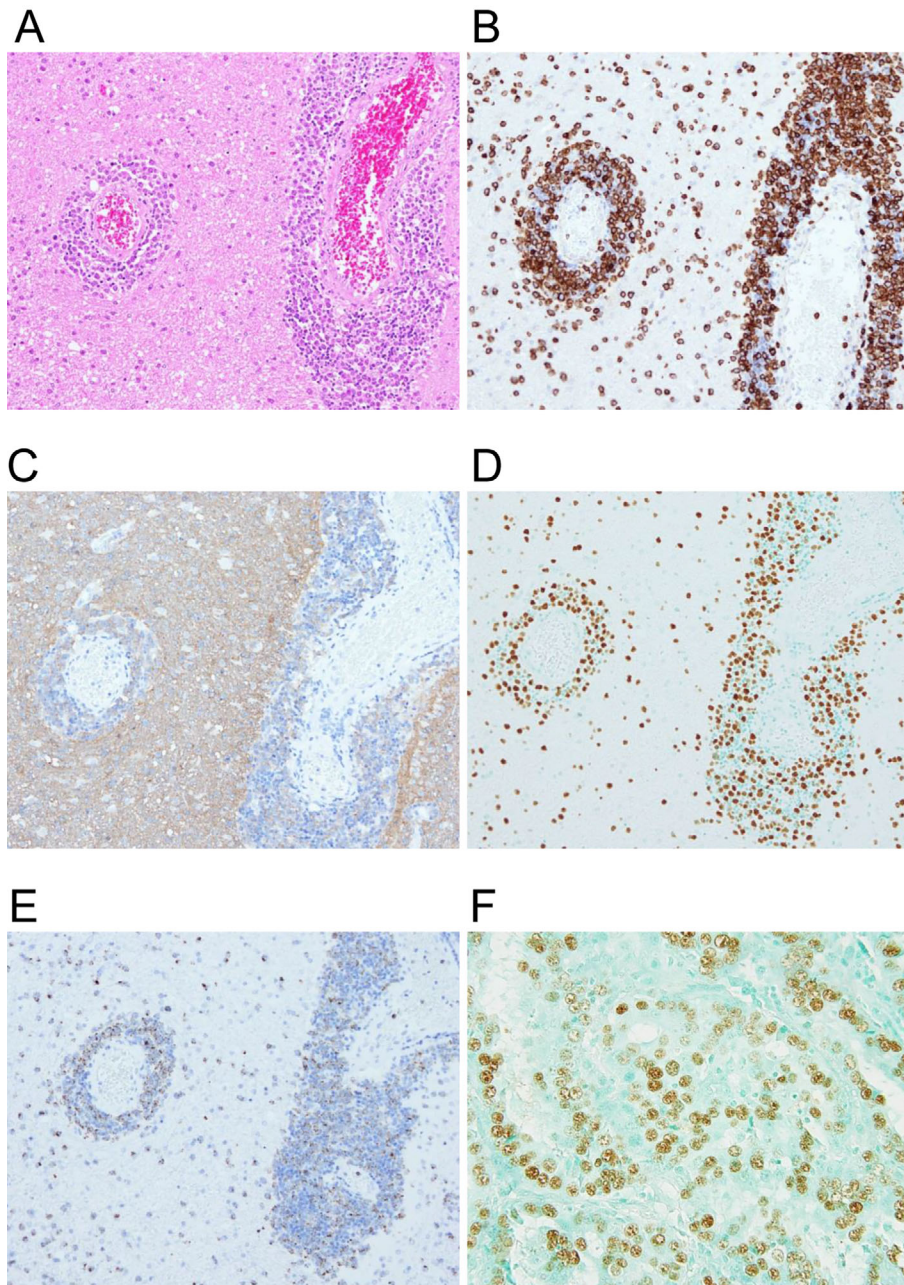


Figure 3. Pathological findings of the CNS lesions. (A) Hematoxylin and Eosin staining. Perivascular infiltration of atypical lymphoid cells observed in the CNS lesion. (B-E) The infiltrating cells were positive for CD3 (B), CD56 (C), Epstein-Barr virus (EBV) (D), and TIA1 (E). EBV was detected by *in situ* hybridization (ISH) of EBV-encoded mRNA (EBER). The original magnification was 1,000 \times . (F) The positive control for EBER-ISH using the specimen from EBV-positive gastric cancer.

were positive for EBV. EBV DNA was negative in the peripheral blood. The DNA extracted from the right vitreous and brain lesions was amplified for the sequence analysis of *LMP1*, an EBV gene, to determine homology. The forward and reverse primers were 5'-GCT GTC GAC GCC ACC ATG GAA CAC GAC CTT GAG AGG-3' and 5'-GCT GGA TCC TTA GTC ATA GTA GCT TAG CTG AAC-3', respectively, and their nucleotide positions in EBV variant B95.8 (Genbank No. V01555) were 169474-169454 and 168160-168183, respectively. This lesion had sequence variations in each EBV (5, 6). The conditions for the PCR reac-

tions were as follows: 94°C for 2 minutes, 98°C for 10 seconds, 62°C for 30 seconds, and 68°C for 90 seconds for 40 cycles. The *LMP1* sequence was confirmed after TA cloning with PCR products. As shown in Fig. 4, the repeat regions of *LMP1* detected in the vitreous and brain lesion were identical. Based on the clinical, pathological, and genetic findings, the CNS lesion was confirmed to be closely associated with and most likely originated from the uveitis. The patient was treated with intravenous high-dose MTX at 3.5 g/m², administered 3 times, and has since maintained CR for 24 months without recurrence.

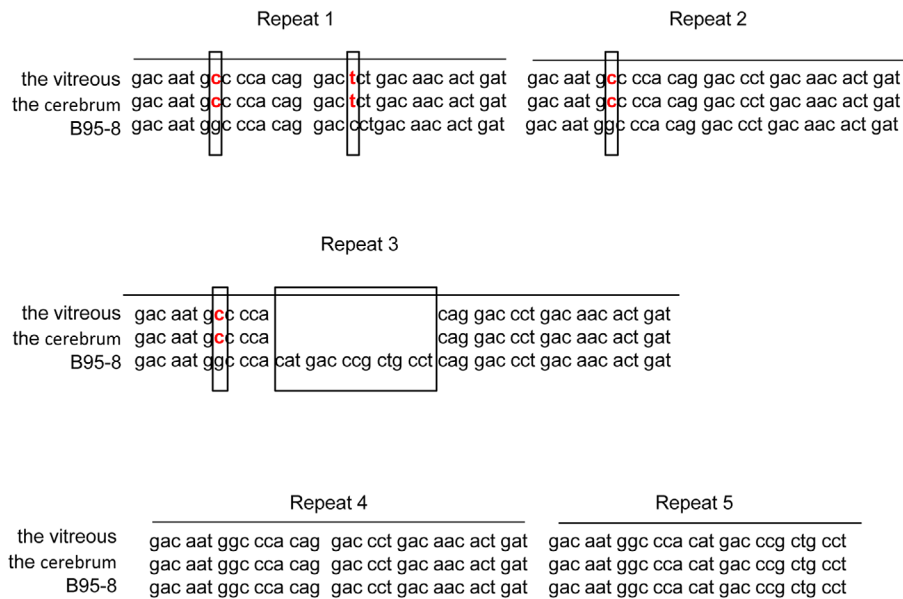


Figure 4. A sequence analysis of *LMP1* from the DNA isolated from the vitreous and the CNS specimen. The sequence of exon 3 of *LMP1* detected in DNA from the vitreous, brain, and B95-8 cells (Accession No. X01995.1) serving as control. The regions bounded by squares indicate regions identical between the vitreous and brain DNA.

Discussion

The ocular findings in this patient were atypical for vitreoretinal lymphoma. First, the gene rearrangements for IgH and TCR were negative. In addition, the ratio of vitreous fluid IL10/IL6 was <1. Finally, the tumor cells were detected neither in the vitreous sample nor in the pathological specimen from enucleation. In contrast, our results indicated a high EBV DNA load in the vitreous fluid. Uveitis can occur in EBV-positive T-cell or NK-cell lymphoproliferative diseases (EBV-T/NK-LPDs), also known as chronic active EBV infection (7). However, this patient could not be with EBV-T/NK-LPDs, as her peripheral blood was negative for the EBV DNA (8, 9).

Although we were unable to diagnose the vitreous lesions as lymphoma, ENKL developed in the CNS, and the virus detected in the ENKL lesion was identical to that found in the vitreous fluid. There are two potential mechanisms for the development of CNS lymphoma in our patient: the transformation of EBV-infected cells infiltrating the uvea, and the metastasis of preexisting lymphoma in the uveal lesion to the CNS. One study demonstrated that while multiple EBV strains could be detectable within one individual, only one strain could induce transformation (10). The detection of the same strain of EBV in the uveitis and the CNS lesion in this case supports the second hypothesis. A pathological diagnosis of vitreoretinal lymphoma is generally difficult due to the challenges in obtaining a sufficiently large specimen and in detecting lymphoma cells in the specimen. Therefore, a molecular diagnosis by PCR for IgH or TCR gene rearrangements is recommended for suspected cases. We failed

to detect clonality in our patient; however, because IgH and TCR gene rearrangements are usually negative in ENKL cases, the clonality of the tumor cells should be determined by an analysis of the terminal repeats of EBV genes.

To date, two cases with primary ocular ENKL lesions have been reported (11, 12). In the case reported by Maruyama et al., the lesion was resistant to steroids; however, intravitreal injection of MTX was effective. In that case, SMILE therapy that included prednisolone, methotrexate, ifosfamide, L-asparaginase, and etoposide was added to prevent CNS disease; however, the outcome was not described (11). In the case reported by Tagawa et al., the IL10/IL6 ratio was <1.0, as in the present case (12). The tumor cells in the vitreous fluid were CD-56- and EBV-positive. The patient passed away due to hemophagocytic lymphohistiocytosis 26 days after the first visit. There have been eight reported cases of ENKL with primary CNS lesions (Table) (13-20). Case 3 reported by Cobo et al. had acquired immune deficiency syndrome (AIDS) (15). None of the cases had EBV-positive uveitis prior to the CNS disease. Three of the 8 cases (Cases 2, 6, and 8 in Table) were treated with systemic high-dose MTX that did not prevent ENKL development; those patients died 2-18 months after diagnosis. In one patient (Case 8 reported by Liao et al.), however, sequential chemotherapy including high-dose MTX (dose not shown) was effective (20). In our patient, high-dose MTX at 3.5 g/m² was also successful in achieving sustained CR for 8 months. The reduction of the tumor mass by resection before high-dose MTX may have enhanced the antitumor efficacy of MTX. Further studies are needed to determine effective treatment strategies for ENKL in the CNS.

Table. Reported Cases of Extranodal NK/T-cell Lymphoma Nasal Type with Primary CNS Lesions.

Case	Gender/Age	Immunological state	Treatment	Outcome (Survival period)	Reference Number
1	M/40	IC	ND	died (7 months)	13
2	M/53	IC	MTX (dose unknown)	died (2 months)	14
3	M/43	AIDS	none	died (1 months)	15
4	M/26	IC	Rx, CVAD+IT (MTX+Ara-C) ESHAP+GEM	died (6 months)	16
5	M/25	IC	Rx, MTX (8.8g/day), TMZ (day1-5)	died (18 months)	17
6	F/73	IC	Rx	died (10 months)	18
7	F/60	IC	Rx, MTX (8g/m ²)	died (3.5 months)	19
8	F/68	IC	MPV, AraC (through Ommaya reservoir), high dose AraC, IT (MTX and AraC)	ND	20

IC: immunocompetent, ND: not described; MTX: methotrexate, AIDS: Acquired Immune Deficiency Syndrome, Rx: radiation therapy, CVAD: cyclophosphamide, vincristine, doxorubicin, dexamethasone, IT: intrathecal, Ara-C: Cytarabine, ESHAP: cisplatin, etoposide, methylprednisolone, cytarabine, GEM: gemcitabine, TMZ: temozolomide, MVP: methotrexate, vincristine, procarbazine

We previously reported the results of screening of ocular infectious diseases in patients with uveitis by multiplex PCR of DNA extracted from the vitreous fluid, where EBV DNA was detected in 27% (17/60) of uveitis cases at our hospital; 3 of those had high EBV DNA loads in the vitreous fluid at 7.3×10^3 - 2.4×10^8 copies/mL (3). Nahdi et al. from Tunisia also reported that EBV DNA was positive in 19% (4/21) of the patients with uveitis, as determined by PCR of the vitreous fluid (21). These results suggest that the detection of EBV DNA in the vitreous fluid may not be rare in uveitis. In addition, 47% (8/17) and 75% (3/4) of EBV DNA-positive patients presented with retinal necrosis in our previous report and Nahdi's study, respectively. The case described in the present study had massive necrosis in the enucleated eye, similar to that observed in reported cases of EBV uveitis. Further studies should be performed to clarify the clinical features of EBV-positive uveitis, especially its relationship with lymphoma.

In conclusion, this is a case of ENKL following EBV-positive uveitis. EBV-positive uveitis should be carefully examined and followed up, even if the vitreous findings are atypical for lymphoma.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

This work was supported by a grant from the Ministry of Health, Labor, and Welfare of Japan (H26-Nanchi-071), a grant from the Practical Research Project for Rare/Intractable Diseases (15ek0109098) from Japan Agency for Medical Research and development, AMED, and a grant from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (15K09468).

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