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CASE REPORT

Immunomodulator-associated Epstein–Barr virus-positive mucocutaneous ulcer in a patient with refractory Crohn's disease

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Abstract
Epstein–Barr virus (EBV)-positive mucocutaneous ulcer is a B-cell lymphoproliferative disorder occurring in elderly or iatrogenic immunocompromised patients. We report a 27-year-old male patient with Crohn's disease (CD) who developed immunomodulator-associated lymphoproliferative disorder. The patient was diagnosed with CD at the age of 17 and was treated with maintenance therapy including high-dose infliximab and azathioprine. When he was admitted to our hospital with a diagnosis of intestinal obstruction, his abdominal computed tomography findings showed not only colonic wall thickening and narrowing of the descending colon but also multiple liver tumor lesions. His ileus symptom improved with conservative therapy, and a pathological evaluation of the tissue biopsy specimens from the descending colon and liver lesions indicated a morphological diagnosis of EBV-positive diffuse large B-cell lymphoma. This was a case of iatrogenic immunodeficiency-associated lymphoproliferative disorder due to an immunomodulator. The treatment was initiated with chemotherapy, but he died of disease progression 10 months after the diagnosis of lymphoma. Although cases of lymphoproliferative disorder due to treatment modalities used for CD are rare in Japan, an increase in the risk of lymphoproliferative diseases should be considered in patients with CD treated with immunomodulatory agents.

Keywords Crohn's disease · Malignant lymphoma · Immunomodulatory agent · Epstein–Barr virus · Epstein–Barr virus-positive mucocutaneous ulcer

Introduction

Lymphoproliferative disorders, including malignant lymphoma (ML), in patients with Crohn's disease (CD) are rarely reported in Japan [1]. We present the case of a young male patient with CD who was diagnosed with ML while he was in remission during maintenance treatment that included immunomodulatory agents and the anti-tumor necrosis factor A antibody. Based on the results of the tissue biopsy specimens from lesions in the descending colon and liver, the patient was diagnosed with Epstein–Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL).

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Case report

A 27-year-old man who had been diagnosed with ileocolic-type CD at 17 years of age was admitted to our hospital with abdominal pain and fever. He did not have any other chronic disorders. The patient's medical history revealed that he was first started on 5-aminosalicylic acid and nutrition therapy with an elemental diet for CD; however, because of the intractable disease course, he was placed on induction of remission therapy with 5 mg/kg infliximab, which was subsequently administered as maintenance therapy at 8-week intervals from the age of 20 years. The patient developed perianal abscess, perforation of small intestine, and small intestinal fistula at the ages of 20, 22, and 23 years, respectively, which were influenced by the loss of response to infliximab; he underwent abdominal surgery in each case. Following the last surgery, his maintenance therapy was changed to short-interval infusion (6 weeks) with 5 mg/kg infliximab. An attempt to switch the patient to adalimumab in combination with azathioprine at the age of 24 years failed due to elevated disease activity within 3 months; the patient had to receive reinduction therapy with infliximab. Thereafter, he remained in remission (CD activity index < 150) with escalation of infliximab dose (10 mg/kg) and azathioprine (100 mg/day) for the next 4–5 years. The mean corpuscular volume consistently ranged from 90 to 100 fl. Endoscopic examination was performed annually for the last 4 years. Although clinical remission was preserved, linear ulcers and stenosis of the descending colon from X-2 developed and gradually became worse. We performed colonoscopy a month before admission. At this time, we managed to pass the colonoscope through the stenosis and linear ulcer of the descending colon. We performed biopsy of the ulcer, but no malignant findings were observed. The clinical course of CD in this patient is summarized in Fig. 1.

Despite having received maintenance treatment, he was admitted to the hospital due to exacerbation of abdominal symptoms when he was 27 years old. A physical examination revealed a body temperature of 37.0 °C, a systolic/diastolic blood pressure of 95/58 mmHg, and a pulse rate of 68 beats/min. He reported one very small bowel movement daily. There were no palpable lymph nodes in the neck, axillary, or inguinal areas. Bowel sounds were somewhat enhanced, with slight abdominal distension; the abdomen, while soft, was tender in the epigastric area. There was no rebound tenderness.

Blood cell count revealed severe anemia, with a hemoglobin concentration of 8.2 g/dL. The white blood cell count was 3900/μL. Other laboratory tests did not indicate significant liver or renal dysfunction or signs of malnutrition, except for a slightly elevated C-reactive protein level

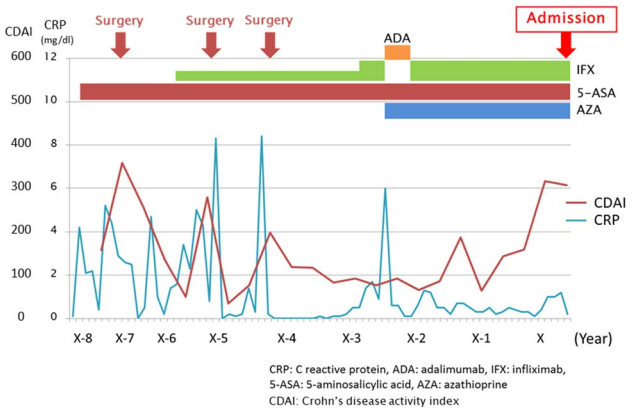

Fig. 1 Clinical course of the patient before admission. The duration of each drug treatment is indicated by a crossbar, and the C-reactive protein levels and Crohn's disease activity index score are presented as a line graph

(0.6 mg/μL). The serum soluble interleukin-2 receptor level was high (3846 IU/μL). Hepatitis B virus, hepatitis C virus, human immunodeficiency virus, and human T-cell lymphotropic virus type 1 were ruled out; however, the patient had been found as subclinical EBV infection. These findings are presented in Table 1.

Abdominal X-ray showed stenosis of the descending colon and expansion and niveau formation in the right colon, extending from the ascending colon to the splenic flexure. No other findings of evident small intestinal expansion or free air were noted. Abdominal contrast-enhanced computed tomography (CT) revealed wall thickening of an area > 15 cm in length, with a pale contrast effect, located in the descending colon; mild lymph node enlargement around the area was also observed. Three ischemic liver lesions were detected in segments 1, 3, and 5, which ranged between 20 and 25 mm in diameter (Fig. 2). These tumors were found to exhibit rapid growth based on comparison with the CT findings from 1 month ago. Positron emission tomography (PET) showed abnormal accumulation of ^{18}F-fluorodeoxyglucose in the descending colon, the regional lymph nodes, and the three liver tumors, with standardized uptake values ranging between 15 and 26 (Fig. 3).

Based on the laboratory and imaging findings, ileus due to constriction associated with wall thickening in the descending colon was suspected. Surgical colonic resection following colorectal stenting was considered, as metastasis of liver cancer in the descending colon was suspected based on PET-CT. However, because the diagnosis was not confirmed by pathologic evaluation and the extent of the inflammation due to CD was not clear, in consultation with the surgeon, the patient was started on conservative medical treatment by nasal insertion of an ileus tube to resolve the ileus instead of emergency surgery,

Table 1 Laboratory data on admission

Complete blood count		Serum chemistry		Infection	
WBC	3900/μL	AST	21 U/L	EBV-EBNA	4.9
Hemoglobin	8.2 g/dL	ALT	8 U/L	EBV EA-DR IgG	×40
Platelet count	38.5×10^4/μL	LDH	194 U/L	EBV-VCA IgM	Negative
		ALP	170 U/L	CMV C7-HRP	Negative
Tumor marker		γGTP	240 U/L	HBs-Ag	Negative
AFP	2.9 ng/mL	BUN	9 U/L	HCV-Ab	Negative
CEA	0.5 ng/mL	Cre	0.84 mg/dL	HIV-Ab	Negative
CA19-9	1.8 U/mL	Na	135 mEq/L	HTLV-1-Ab	Negative
sIL-2R	3846 U/mL	K	3.8 mEq/L		
		CRP	0.6 mg/dL		

WBC white blood cell, *AFP* alpha-fetoprotein, *CEA* carcinoembryonic antigen, *CA19-9* carbohydrate antigen 19-9, *sIL-2R* soluble interleukin-2 receptor, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *LDH* lactate dehydrogenase, ALP alkaline phosphatase, *γGTP* γ-glutamyltransferase, *BUN* blood urea nitrogen, *Cre* creatinine, *Na* sodium, *K* potassium, *CRP* C-reactive protein, *EBV* Epstein–Barr virus, *EBNA* Epstein–Barr virus nuclear antigen, *EA* early antigen, *VCA* virus capsid antigen, *CMV C7-HRP* cytomegalovirus pp65 antigen test, *HBs-Ag* hepatitis B surface antigen, *HCV-Ab* anti-hepatitis C virus antibody, *HIV-Ab* anti-human immunodeficiency virus antibody, *HTLV-1-Ab* anti-human T-cell lymphotropic virus type 1 antibody

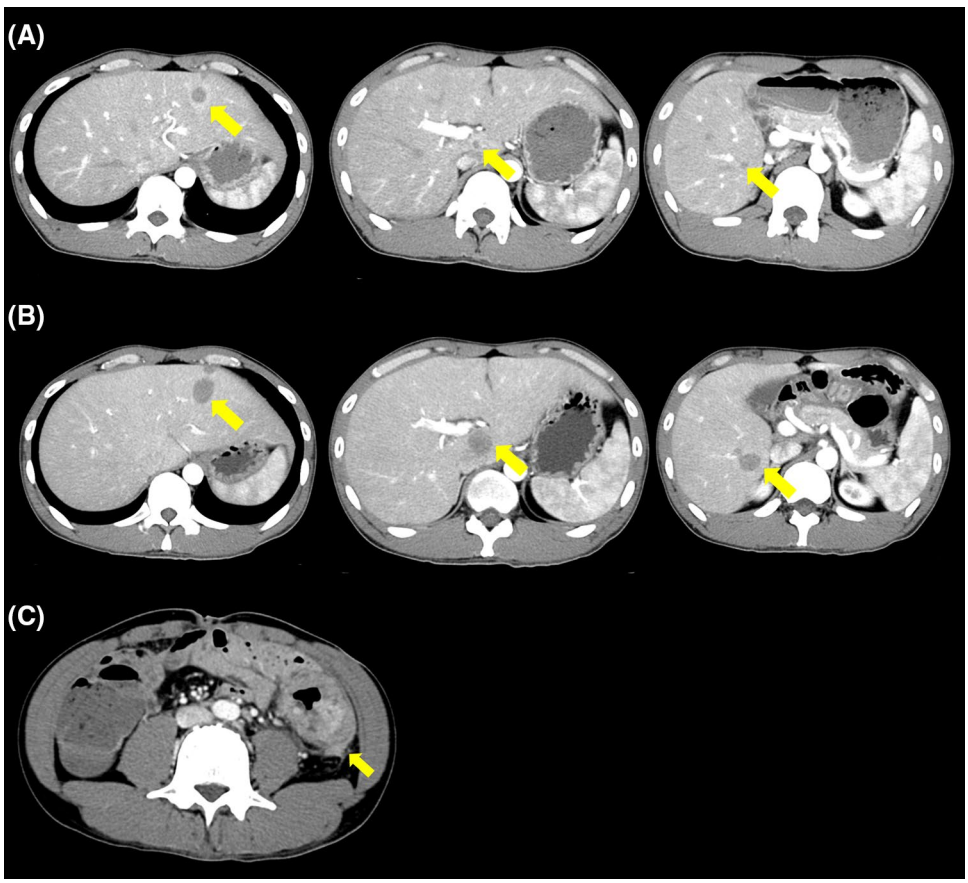

Fig. 2 Abdominal contrast-enhanced computed tomography. Liver lesions during the arterial phase (indicated by arrows) 1 month before admission (**a** upper) and on admission (**b** lower). The lesions rapidly increased in size in a short time interval. **c** Wall thickening of the descending colon on admission

while further diagnostic evaluation was conducted. After admission, all CD drugs, including biologics and immunomodulatory agents, were discontinued.

The ileus tube was inserted on the fourth hospital day, and despite poor drainage, the patient resumed natural bowel movements, with concurrent natural improvement of ileus.

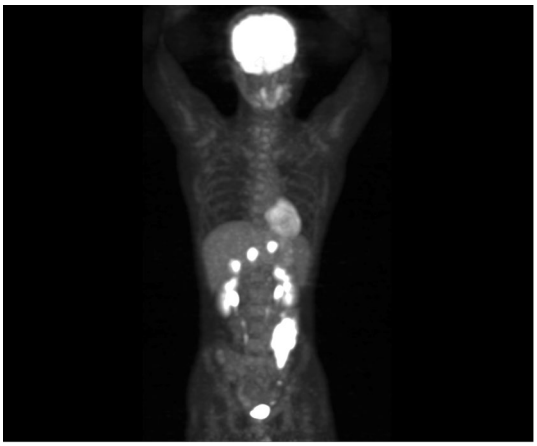

Fig. 3 Findings of positron emission tomography. Abnormal accumulation of ^{18}F-fluorodeoxyglucose was seen in the descending colon, the regional lymph nodes, and the three liver tumors, with standardized uptake values ranging between 15 and 26

Ileus was successfully managed without relapse by fasting and fluid replacement. Based on imaging studies, the differential diagnosis included metastasis of liver cancer in the descending colon, ML, inflammatory pseudotumor, and liver abscess with exacerbation of CD; however, due to the difficulty of reaching a diagnosis based on imaging, the patient underwent endoscopy for colonic mucosa biopsy on the seventh hospital day and liver tumor biopsy on the nineteenth hospital day for pathologic diagnosis.

Colonoscopy showed two giant deep ulcers throughout the lumen of the descending colon. The colonoscope could not pass through the proximal side of the colon because the edge of the ulcers was rising steeply. Based on these findings, epithelial tumors were initially suspected (Fig. 4). The endoscope could not be advanced beyond the stenotic area. Biopsies were performed on these ulcers. Ultrasound-guided liver biopsies were also performed on lesions in segment 3 by a 16-gage Monopty needle. The pathologic findings of the descending colon biopsy specimens were similar to those of the liver biopsy specimens. Hematoxylin and eosin staining revealed diffuse proliferation of large lymphoid cells with prominent nuclear atypia. Immunostaining showed that the specimens were partially positive for CD20 and the high Ki-67 labeling indexes of the liver lesions and the descending colon were approximately 50% and 90%, respectively. All biopsy specimens were positive by EBV-encoded RNA (EBER) in situ hybridization (ISH). Bone marrow biopsy showed no evidence of tumor invasion. Therefore, the patient received a morphological diagnosis of clinical stage 4A EBV-positive DLBCL, an iatrogenic immunodeficiency-associated lymphoproliferative disorder, with EBV-positive mucocutaneous ulcer (Fig. 5). Based on the diagnosis, cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) therapy was initiated in combination with rituximab (R-CHOP) in the Hematology Department. A reduction in the liver lesion size was observed, and the obstruction of the digestive tract was improved after the first course. He was discharged with maintenance of oral ingestion of an elemental diet. The patient experienced disease progression after five courses of R-CHOP therapy and underwent salvage treatment followed by high-dose chemotherapy and autologous stem cell transplantation, but the disease became refractory. He died of disease progression 10 months after the diagnosis of lymphoma.

Discussion

The increased incidence of lymphoproliferative disorders due to long-term inflammation and use of immunosuppressive drugs in patients with inflammatory bowel disorders (IBDs), including CD, remains a concern; however, data on actual rates are limited. In Japan, a nationwide questionnaire survey by Fukata et al., which included 70 facilities and 6810 patients with CD, reported that lymphoproliferative disorders, including ML, leukemia, multiple myeloma, and myelodysplastic syndrome, were diagnosed in 16 patients (0.23%), with five cases of ML [1]. In that survey, only one patient was treated with oral immunomodulatory agents. Therefore, immunomodulator-associated ML

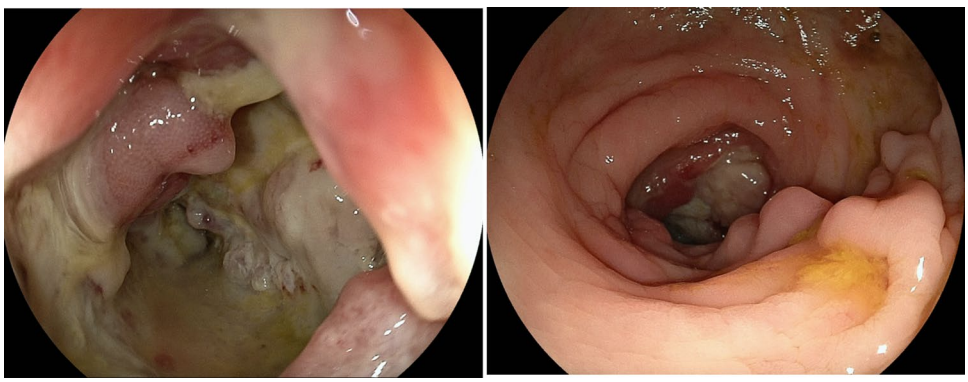

Fig. 4 Colonoscopy showing deeply eroding ulcers of the descending colon. The endoscope could not be advanced beyond the stenotic area

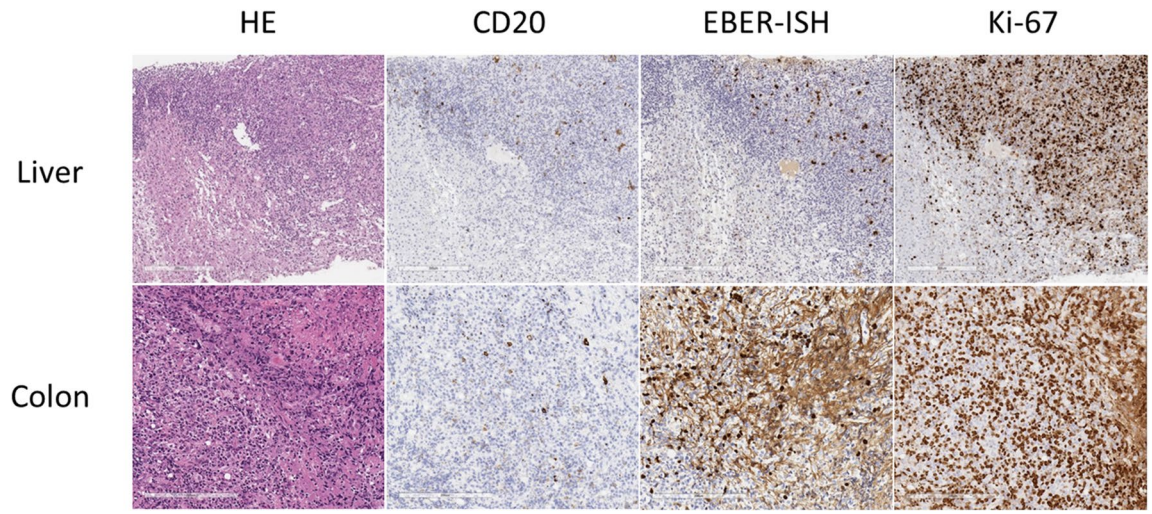

Fig. 5 Pathologic assessment of biopsy specimens from the descending colon and liver lesions. The specimens were positive for CD20 and Ki-67 by immunostaining and for Epstein–Barr virus-encoded RNA by in situ hybridization

in patients with refractory CD, such as the current patient, may be very rare in Japan.

The pathological classification of post-transplantation lymphoproliferative disorders (PTLD) by the World Health Organization (WHO) describes the continuum of lymphoproliferative disorders treated by immunosuppressive therapy [2]. The use of immunomodulatory agents with concurrent EBV infection was previously reported as a risk factor for lymphoproliferative disorders after liver transplantation [3]. EBV induces B-cell transformation and proliferation through complex mechanisms. Viral gene products upregulate a variety of cellular antigens and the expression of genes in B cells. Activation of key molecular pathways controlling the cell cycle, such as NF-kB and virus-induced cytokines, exerts proliferative effects in a paracrine manner [4, 5]. Stephan et al. designated the lesions observed in EBV-associated lymphoproliferative disorders, which had significantly higher EBER expression levels than normal lymphoid tissues by in situ hybridization, as EBV-associated mucocutaneous ulcers (EBV-MCUs) [6]. Most EBV-MCUs caused by immunosuppressive therapy were reported to undergo complete remission only after immunosuppression was reduced; however, the current case exhibited a malignant clinical course, highlighting the potential adverse outcomes of EBV-MCUs. There are some methods of detection of EBV in tissue, such as immunohistochemical staining for EBV-determined nuclear antigen, latent membrane protein-1, polymerase chain reaction for the gene of EBV, and EBER-ISH. We considered the EBER-ISH method extremely useful in combination with tissue diagnosis, including not only EBV detection but also evaluation of the infection status of the virus at the tissue or cellular level.

Several studies have reported increased incidence rates of lymphoproliferative disorders such as ML due to thiopurine use in patients with IBD. In a prospective cohort study, Beaugerie et al. reported that the hazard ratio for lymphoproliferative disorders in thiopurine-treated IBD patients compared with thiopurine-naïve patients, was 5.28, a significant difference [7]. Kotlyar et al. reported that the standardized incidence ratio (SIR) of lymphomas associated with IBD due to the use of thiopurine was 4.493 [8]. Because it has been reported that administration of infliximab and azathioprine in combination for active CD is preferable to infliximab monotherapy [9], there is concern that the number of cases of iatrogenic lymphoma will increase in the future.

Whether IBD itself is a risk for lymphoproliferative disorders remains unclear at this stage. Askling et al. analyzed prospective data from nearly 50,000 Swedish patients with IBD [10]. They calculated SIRs based on lymphoma rates in this cohort in comparison with the expected rates in the general population, and reported a slight but significant increase in lymphoma risk in patients with CD (SIR = 1.3), especially in those who required hospitalization. Although several other studies also reported that patients with CD were at increased risk for hematological cancer [11–13], other groups reported contradictory findings [14, 15]. According to the WHO classification of ML, MLs due to chronic inflammation such as chronic empyema and osteomyelitis are described as chronic inflammation-related DLBCL [16]. Therefore, long-term chronic inflammation in patients with CD may be associated with the development of inflammation-related ML.

This study has some limitations. EBV-DLBCL of people aged 50 years or older is classified as one disease, "EBV-DLBCL in elderly", in the 2008 WHO

classification. The prevalence rate is reported to be 8–15% in Asia and less than 5% in Western countries [17]. Many cases of developing DLBCL are reported even in people younger than 50 years, despite the absence of immunodeficiency [18–20], so it is difficult to conclude that drug-induced immunosuppression was the cause of onset in the present case. However, it is undeniable that long-term immunosuppressive therapy has some influence on the development of lymphoma because immunodeficiency is often involved in the development of lymphomas such as methotrexate-related DLBCL or PTLD. It is known that EBV-associated lymphoproliferative disease occurs under immunosuppression [6], although it is unclear if an immunosuppressive state is directly involved in the development of LPD. Moreover, EBER-ISH is one of the most important techniques for the detection of EBV infection pathologically and was previously performed by Dojcinov et al. to reveal that EBV-associated lymphoproliferative disease was associated with EBV infection in the tissue. Although EBV-MCUs might not have the same etiology as EBV-DLBCL, we concluded that this case was an iatrogenic EBV-DLBCL due to thiopurine use as well as lymphoma due to methotrexate use in the WHO classification.

Here, we report a patient with CD who developed EBV-associated ML. The clinical course of the patient suggested the involvement of immunomodulatory agents in the development of ML. The current case, together with previously reported cases, highlights the risk of lymphoproliferative disorders due to the long-term use of immunomodulatory agents for IBD.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interest.

Ethical approval This report was reviewed and approved by the Chiba University Ethical Committee.

Human and animal rights This article does not contain any studies directly involving human participants, as it is a review of data already collected in a hospital database.

Informed consent For this type of study, formal consent was not necessary.

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