Primary Gastric T Cell Lymphoma with Epstein-Barr Virus Infection.

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ABSTRACT

Primary gastric T cell malignant lymphomas are very rare. Here we report a case of primary gastric T cell lymphoma infected with Epstein-Barr virus (EBV). Histologic and immunohistologic features, positive for CD45RO/UCHL1 and negative for CD20/L26, showed this tumor to be a diffuse, large cell type, malignant lymphoma with a T cell phenotype. Genotypic analysis by the Southern blot method, demonstrating T cell receptor gene rearrangement and a germline configuration of the immunoglobulin gene, also supported the T cell origin of this tumor. EBV DNA was detected by polymerase chain reaction and in situ hybridization against EBER1 demonstrated that EBV was located in the lymphoma cells. This is the first Japanese case report of primary gastric malignant lymphoma of T cell origin infected with EBV.

INTRODUCTION

Most malignant tumors arising in the stomach consist of adenocarcinomas of epithelial origin. Those of non-epithelial origin, which consist mainly of malignant lymphomas and leiomyosarcomas, are rare. Among such rare cases of gastric malignant lymphomas, those of T cell origin are extremely rare (1, 2, 3, 4, 5, 6), and Epstein-Barr virus (EBV)-infected primary gastric T cell lymphoma has not, prior to this account, been reported in Japan.

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EBV infects oropharyngeal epithelium and B-lymphocytes and is known to be associated with nasopharyngeal carcinoma, endemic Burkitt's lymphomas and the lymphoproliferations that occur in immunosuppressed patients (7, 8). Significant relationships between EBV and other types of neoplasias such as T cell type non-Hodgkin lymphomas (9, 10, 11), Hodgkin's disease(12), nasal T cell lymphoma (13), AILD type T cell lymphoma (14), lymphomatoid granulomatosis (15), salivary gland tumors (16), and lung tumors (17) have also been suggested. A relationship with gastric cancers was also demonstrated by polymerase chain reaction (PCR) and in situ hybridization, suggesting EBV infection to be causative of gastric cancers (18, 19, 20, 21). On the other hand, the mechanism of EBV infection of various types of T cell lymphomas and involvement of EBV in the pathogenesis of these tumors remain uncertain. Here we report a rare case of primary gastric T cell lymphoma with EBV, which is a suspected carcinogenic agent in gastric cancer.

CASE REPORT

A 58-year-old man, suffering from diabetes mellitus, visited a clinic with general fatigue and epigastralgia. Endoscopic examination showed a multiple ulcer and broad swelling of the gastric wall with giant folds. A biopsy suggested a malignant tumor, though a definite diagnosis was not made. He was admitted for detailed examination and treatment. On admission he showed no evidence of generalized lymphadenopathy or hepatosplenomegaly. Laboratory investigations revealed a slight leukocytosis (9×10^9/L), with mild eosinophilia (7.5%), and urinary sugar with no other significant abnormality. Endoscopical mucosal resection was performed and a diagnosis of malignant lymphoma of the diffuse large cell type was made. Computed tomography and ultrasonographic examinations showed no other tumor signs and this tumor was considered to be of stomach origin. No atypical cells were observed in the bone marrow and the antibody titer against human T cell lymphotropic virus type I (HTLV-I) was negative. A total gastrectomy with splenectomy was performed and at the time of surgery no evidence of liver metastasis or lymph node swelling was found. The patient was subsequently treated with chemotherapy and has been in remission for 5 months.

MATERIALS AND METHODS

Histologic examination ;

Fresh tissue was fixed in 10% formalin and embedded in paraffin. Three-micron sections were made and stained with hematoxylin and eosin.

Immunohistochemical examination ;

Paraffin sections were stained by the avidin-biotin immunoperoxidase complex technique with a VECTASTAIN ABC KIT (Vector Laboratories, Burlingame, USA). Monoclonal antibodies used in this study included UCHL1 (CD45RO), Vimentin (Nichirei, Japan), MT1 (CD43 ; MILAB, Sweden), L26 (CD20 ; DAKO Japan, Japan), LeuM1 (CD15 ; Becton Dickinson, CA), BerH2 (CD30), S100, LMP1, BZLF1 and EBNA2 (DAKO, Denmark).

Genotype analysis ;

Fresh tissue was used for Southern blot analysis. DNA extraction, restriction endonuclease digestion, blotting to the membrane and hybridization were performed according to the standard protocol described elsewhere (14). Cβ1 was used to detect T cell receptor (TCR) gene rearrangement and JH was used to analyze immunoglobulin (Ig) gene rearrangement.

Polymerase chain reaction ;

Oligonucleotide primers were chosen within internal repeat I of EBV DNA and synthesized on an Applied Biosystems Model 391 (Applied Biosystems Inc., Foster City, USA). Thirty cycles of amplification were carried out using a DNA Thermal Cycler (Perkin Cetus, Norwalk, USA). Conditions were described in detail previously (14).

In situ hybridization ;

A synthetic oligonucleotide probe for EBER1 was 3'-end labeled with digoxigenin-dUTP using a DIG oligonucleotide Tailing Kit (Boehringer Mannheim Yamanouchi, Tokyo, Japan) and three-micron- thick paraffin sections on silane-coated slides were processed for in situ hybridization as described previously (22).

Double staining ;

Three-micron-thick paraffin sections on silane-coated slides were stained with an anti-CD4 monoclonal antibody (Novocastra, UK). The sections stained with anti-CD4 were processed for in situ hybridization as described above.

RESULTS

Histologic findings ;

Resection of the stomach showed thick folds throughout with multiple shallow ulcers, edges of which were clear-cut but not raised or rolled (Fig. 1). The neoplastic cells infiltrated the lamina propria mucosae and the submucasal area

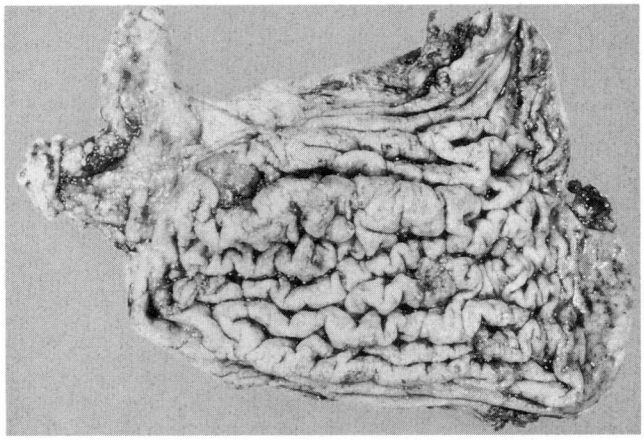

Fig. 1 Gross appearance of the stomach showing extensive thick folds along the lesser curvature and some ulcerations 2 cm in diameter or less.

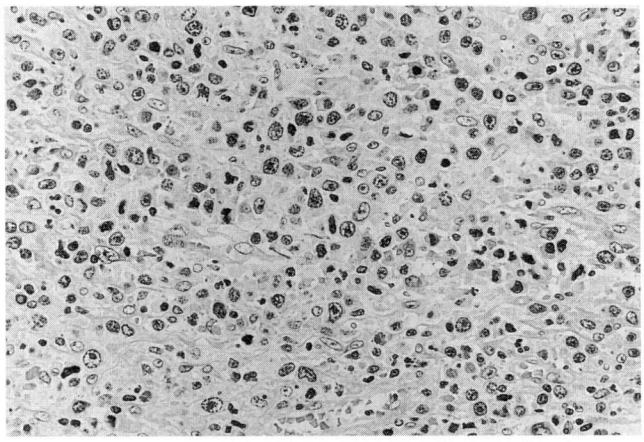

Fig. 2 The lymphoma cells are uniform and large. Eosinophil infiltration is also observed (HE, x400).

with marked submucosal edema but not the muscle. The lymphoma cells consisted of relatively uniform, predominantly large lymphoid cells, which contained a few prominent nucleoli within oval or occasionally convoluted nuclei (Fig. 2). Eosinophil infiltration was observed throughout the tumor area. No lymphoma cells could be found in the spleen. The tumor was thus diagnosed as large cell lymphoma of the stomach.

Immunohistochemistry ;

Results of immunohistochemical staining, positive for UCHL1/CD45RO and

MT1/CD43, negative for L26/CD20, supported the belief that this tumor was of T cell origin. Antigens for anaplastic lymphoma, BerH2/CD30 and LeuM1/CD15 were both negative. Markers for mesenchymal origin, vimentin and S100 were negative and EBV-related latent membrane antigen-1 (LMP1), BZLF1 and EBNA2 were also negative.

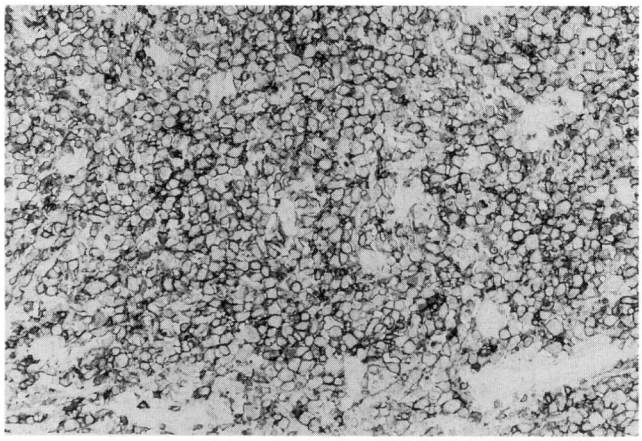

Fig. 3 UCHL1/CD45RO staining demonstrates positive reactivity on the lymphoma cell membranes (x400).

Table 1 Immunohistochemical findings

Antibody	Reactivity
UCHL1/CD45RO	+
MT1/CD43	+
L26/CD20	−
BerH2/CD30	−
LeuM1/CD15	−
S100	−
Vimentin	−
LMP1	−
BZLF	−
EBNA2	−

Genotype analysis ;

Rearrangement of the TCR gene was detected by Southern blotting as shown in Figure 4, but the Ig gene was not rearranged. These results, together with phenotypic analysis, indicated this tumor to be of T cell origin.

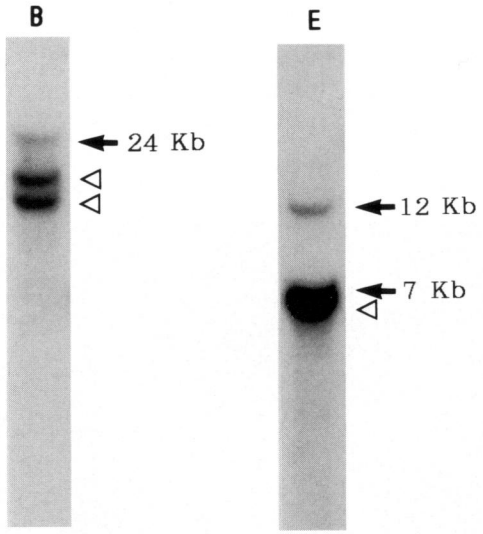

Fig. 4 TCRβ gene rearrangement is detected. B: BamHI, E: EcoRV, ◄: Germline, ◁: Rearranged band

Detection of EBV ;

PCR was conducted to detect internal repeat 1 of the EBV genome in paraffin-embedded sections. A single sharp band of expected length, 129bp, was detected as shown in Figure 5. ISH studies were also performed on paraffin-embedded tissue sections. Dense signals of EBER1 positive cells were observed as shown in Figure 6.

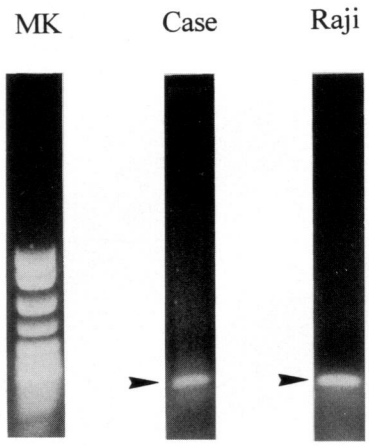

Fig. 5 Amplified DNA of EBV was observed at 129bp (arrowhead). Raji, an EBV-positive lymphoma cell line, was used as a positive control. MK: DNA size marker, pBR322 MspI digest.

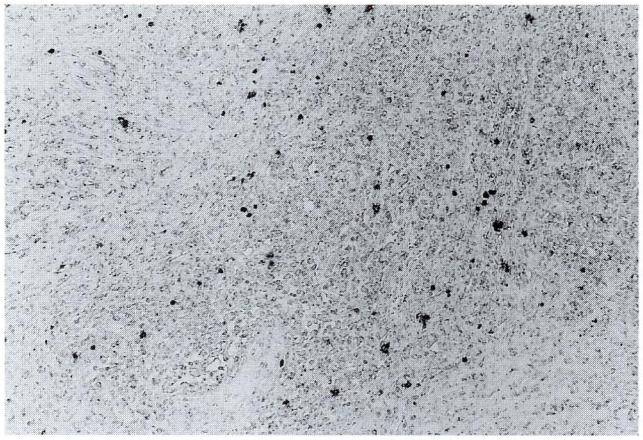

Fig. 6 A positive reaction is seen in the nuclei of tumor cells (x200).

Double staining ;

Immunostaining for the T cell marker and ISH for EBER1 were performed. As shown in Figure 7, some CD4 positive tumor cells were also positive for EBER-1.

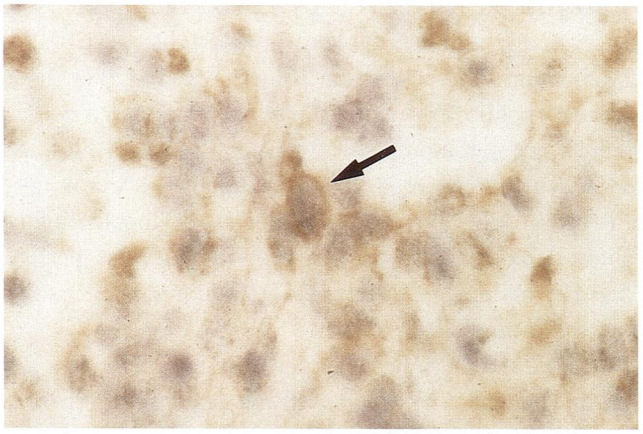

Fig. 7 Double-positive reactions for surface membrane T cell marker CD4 and EBER1 in the nuclei were demonstrated (arrow, x400). The cell membrane is stained brown, and the nucleus is stained gray.

DISCUSSION

Primary T cell lymphomas presenting themselves in the gastrointestinal tract are rare and we were able to find only one report of T cell origin lymphoma associated with EBV infection in the literature which was investigated for EBV

endemic population, (Hong Kong Chinese) (23). Several reports have described primary gastric T cell lymphoma of Japanese cases. Most cases demonstrated the T cell phenotype by immunostaining; however, a few cases were confirmed genotypically. Furthermore, there was no report describing infection by EBV. The first Japanese case report of T cell lymphoma in the stomach was in 1980. Akatsuka reported diffuse mixed cell type lymphoma of stomach origin (24). This case was concluded to be T cell lymphoma by surface phenotype, though no detailed analysis was performed. Mohri reported two cases of gastric T cell lymphoma in an extensive statistical study of extranodal lymphomas (25). However, no evidence of T cell origin was demonstrated. By precise phenotypic study using frozen sections, Nozawa demonstrated a case of the helper/inducer subtype of T cell lymphoma originating in the stomach (5). Genotypic analysis for diagnosis of one case was performed by Kurihara, and rearrangement of the TCR-β gene was detected by Southern blotting (3). Narita also reported three cases of gastric T cell lymphomas presenting T cell surface markers; however, only one case was confirmed to have T cell monoclonality by Southern blotting (26). Two reports demonstrated viral infection in gastric T cell lymphomas; Kubonishi (27) and Yatabe (6) showed monoclonal integration of human T cell leukemia virus type-I (HTLV-I), but no report was found describing EBV infection. This is thus the first Japanese case report of primary gastric T cell lymphoma, phenotypically and genotypically, which showed EBV infection.

Malignant lymphomas of T cell origin sometimes show non-neoplastic cellular elements such as neutrophils, eosinophils, histiocytes, plasma cells, and blood vessels. These findings are commonly seen, especially in IBL-like and AILD-like T cell lymphomas, and are considered to be a response to cytokines elaborated by the neoplastic T cells. The current case showed eosinophil infiltration into the stomach and slight leukocytosis with mild eosinophilia in peripheral blood, so, based on this and the histologic features, we diagnosed this case as peripheral T cell lymphoma of the large cell type.

In the present study EBV infection of lymphoma cells was demonstrated by in situ hybridization and also by double staining; however these procedures did not show LMP1, BZLF1 or EBNA2. These results suggested that the infection by EBV did not correlate with the proliferation of lymphoma cells but that it occurred after the lymphoma was established. However, very little is known about the mechanism of EBV infection of T cell lymphomas. EBV infects B cells through CD21. In addition to B cells, CD21 is expressed on some T cell leukemia lines, immature thymocytes and peripheral blood T cells (28). Kanavaros detected EBV DNA frequently in CD30-positive non-Hodgkin's lymphomas and considered that CD30 expression was induced by EBV rather

than that CD30 played a role as one of the receptor structures (29). Recently, Yoshiyama successfully infected a T cell leukemia line with recombinant EBV to form latency II-type EBV infection (28). This system may be a good model for investigating the role of EBV in such T cell lymphomas. The incidence of EBV-positive T cell lymphomas may not be a rare event in the extranodal lymphomas; as described elsewhere (28). Further studies, including clinicopathological and molecular approaches, are thus required to establish its true frequency and significance.

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