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Case report

Colonal monomorphic epitheliotropic intestinal T-cell lymphoma with novel phenotype of cytoplasmic CD3 expression

Yumi Aoyama,[1)] Hiroko Tsunemine,[1)] Yuriko Zushi,[2)] Hayato Maruoka,[3)] Yuta Goto,[1)] Taiichi Kodaka,[1)] Tomoo Itoh,[4)] Takayuki Takahashi[1)]

Monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL) is a new clinical entity that was reclassified from enteropathy-associated T-cell lymphoma in the 2016 WHO classification. An 83-year-old man with fever and diarrhea was referred to our hospital because of free air in the abdominal cavity and wall thickening of the large intestine on CT. Colonofiberscopic examination revealed mucosal edema and multiple ulcers at the sigmoid colon, splenic flexure, and transverse colon. Histopathological examination of the mucosal biopsy specimen demonstrated dense infiltration of small lymphocytes with nuclear atypia, some of which exhibited intraepithelial invasion. Immunohistologically, these lymphocytes were positive for CD3, CD56, and perforin. Regarding CD3 expression, the antigen was found to only be expressed in the cytoplasm and not on the surface membrane on flow cytometric analysis. PCR examination of the T-cell receptor (TCR) gene revealed monoclonal gene rearrangements of TCR-γ and TCR-β. Based on these findings, a diagnosis of colonal MEITL with cyCD3 expression at Lugano clinical stage 1 was made. After conservative management of the peritonitis, we treated the patient with CHOP and DeVIC regimens, but he developed progressive disease and died. The cyCD3 expression in MEITL may be novel, suggesting a thymocyte origin of the tumor cells.

Keywords: monomorphic epitheliotropic intestinal T-cell lymphoma, cytoplasmic CD3, flow cytometry, thymocyte

INTRODUCTION

Monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL) is defined as a tumor derived from intestinal epithelial T cells. MEITL is a new clinical entity that was reclassified from enteropathy-associated T-cell lymphoma (EATL) in the 2016 WHO classification because of the lack of association with celiac disease.[1] MEITL develops more frequently in Asians than Caucasians, who are mainly affected by EATL. The phenotype of MEITL is different from that of EATL; MEITL is usually positive for CD3, CD8, CD56, and TCR-γδ, but negative for CD30, CD103, and TCR-αβ, with small to medium-sized cells.[1] On the other hand, the majority of EATL cases are positive for cytoplasmic (cy) CD3, CD30, CD103, and TCR-αβ, but are negative for CD8, CD56, and TCR-γδ, with large-cell tumor cells. MEITL is a rare disease with a frequency of less than 5% among primary gastrointestinal malignant lymphomas and comprises 0.25% of all malignant lymphomas in Japan.[2,3] The median age of onset is 60 years old, with no sex-based difference. The primary lesion of MEITL has been reported to be restricted to the small intestine; however, colon-originating MEITL is not rare.[4,5] Clinical features of MEITL are characteristic of multiple ulcerative lesions in the intestinal mucosa without tumor formation. The majority of MEITL cases also have a risk of intestinal perforation. As most cases of MEITL are found in a situation necessitating urgent surgical management, these tumors are generally histopathologically diagnosed with resected or biopsied mucosal materials. Regarding CD3 staining by immunohistopathological methods, CD3 positivity exhibits a similar histopathological pattern regardless of surface membrane (sm) or cytoplasmic (cy) CD3 expression. Therefore, it is unclear whether CD3 positivity reflects sm or cy expression on immunohistopathology. However, flow cytometry (FCM) is able to differentiate smCD3 and cyCD3. Only a few studies on phenotypic analysis of MEITL with flow cytometry have been performed, presumably because of the rapid onset of this disease. We treated a colonal MEITL

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[1)]Departments of Hematology, [2)]Cell Therapy, Shinko Hospital, [3)]Department of Laboratory Medicine, Kobe City Medical Center General Hospital, [4)]Department of Diagnostic Pathology, Kobe University Graduate School of Medicine, Kobe, Japan.
Corresponding author: Takayuki Takahashi, Department of Hematology, Shinko Hospital, 4-47, Wakihama-cho, 1-chome, Chuo-ku, Kobe 651-0072, Japan. E-mail: takahashi.takayuki@shinkohp.or.jp

patient in whom lymphoma cells expressed cyCD3 but not smCD3 based on FCM. We herein report this MEITL case because of the novel phenotype of cyCD3 expression and possible thymocyte origin of the lymphoma.

CASE REPORT

An 83-year-old man with fever and diarrhea was referred to our hospital because of free air in the abdominal cavity and wall thickening of the large intestine on CT. For past medical history, he had had hypertension, diabetes mellitus, and insomnia. For past surgical history, he had undergone polypectomy, cholecystectomy, and management for anal fistula and perianal abscess. He had no particular family history. On admission, he was febrile (38.2°C) and had frequent diarrhea. Physically, he had tenderness of the lower left quadrant with peritoneal signs, but no tumoral lesions were palpable. Neither superficial lymph node swelling nor hepatosplenomegaly was noted.

Serum concentrations of C-reactive protein and soluble IL-2 receptor were elevated to 2.8 mg/dL (normally below 0.3 mg/dL) and 1,537 U/mL (normally below 550 U/mL), respectively. Serum levels of total protein and albumin were decreased to 6.0 and 3.0 g/dL, respectively. Other blood tests were nonspecific, except for mild anemia (12.6 g/dL hemoglobin concentration). No abnormal cells were found in the bone marrow, but chromosomal analysis revealed inv4 and -Y in all 20 and 16 cells analyzed, respectively (data not shown).

Abdominal CT demonstrated wall thickening of the large intestine at the transverse colon, splenic flexure, and sigmoid colon without tumoral lesions (Figure 1). The free air observed at the previous hospital had disappeared on admission to our hospital. Mucosal edema of the entire wall and multiple ulcers at the portions indicated by CT were observed on colonofiberscopic examination (Figure 2). Histopathological examination of the mucosal biopsy specimen indicated dense infiltration of small lymphocytes with nuclear atypia, some of which exhibited intraepithelial invasion. Immunohistologically, these lymphocytes were positive for CD3, CD56, and perforin, and partially positive for granzyme B (Figure 3), but negative for CD4, CD5, CD8, T-cell intracytoplasmic antigen (TIA)-1, and Epstein-Barr virus (EBV)-encoded small RNA (EBER) (data not shown). Regarding CD3 expression, the antigen was found to be expressed only in the cytoplasm and not on the surface membrane by FCM analysis (Figure 4), whereas the smT-cell receptor (TCR)αβ, smTCRδγ, and cyperforin were negative by this analysis. The sole cyCD3 expression was the same by FCM analysis on repeat colonofiberscopy. In this study, we employed anti-CD3 monoclonal antibodies for immunohistochemistry (Roche Diagnostics Co Ltd. Tokyo, Japan) and FCM (Becton, Dickinson and Company, Tokyo, Japan), respectively. Both antibodies recognize the main component of CD3, CD3ε. We considered these abnormal lymphocytes to be of T-cell or natural killer (NK) cell nature; however, PCR analysis of the TCR gene demonstrated monoclonal gene rearrangements for both TCR-γ and TCR-β (Figure 5). This suggested that these abnormal lymphocytes were an αβ-type T-cell tumor. Chromosomal analysis of the colonal mucosa yielded no splitting cells, and no amplification of 8q24 (c-myc) or 7q31 (c-met), which has been reported to be occasionally detectable in MEITL, was found on fluorescence in situ hybridization (FISH) analysis. Multiplex viral PCR analysis[6] of peripheral blood and colonal mucosa detected the EBV genome (2.6×10^2 copies/mL and 1.8×10^4 copies/μg DNA, respectively). Based on these findings, a diagnosis of colonal MEITL with cyCD3 expression at Lugano clinical

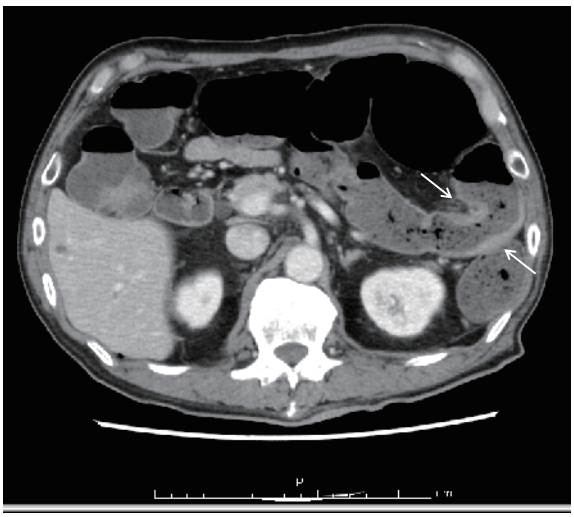

Fig. 1. Abdominal contrast CT on admission. Wall thickening of the large intestine at the transverse colon (arrows) without tumor formation was seen.

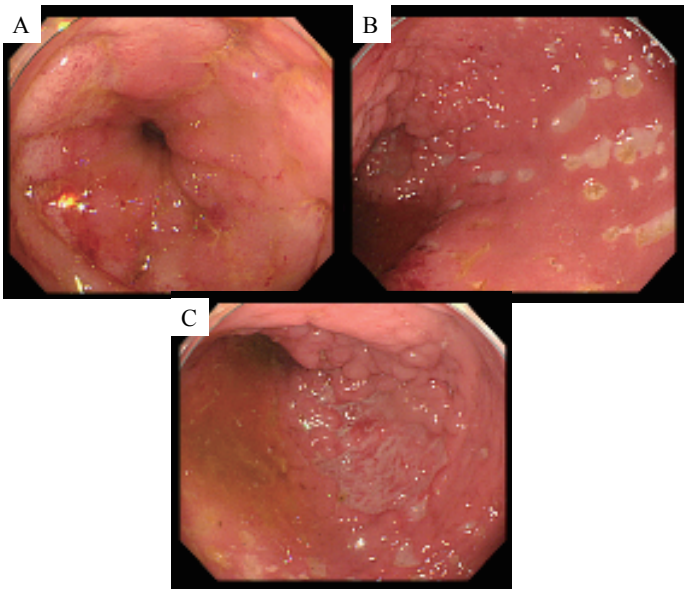

Fig. 2. Colonofiberscopic examination on admission. ***A***: Mucosal edema and redness of the entire wall at the regions indicated on CT; ***B***: multiple small ulcers in the same regions; ***C***: A map-like ulcer in the sigmoid colon.

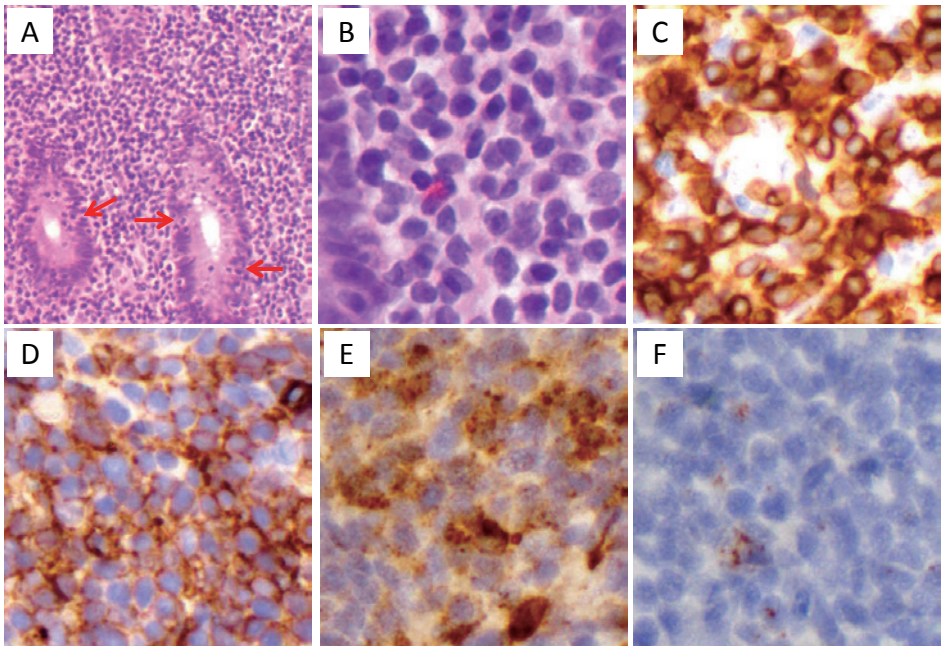

Fig. 3. Histopathological examination of a biopsy sample from the mucosa lesion in the colon. ***A***: Dense infiltration of small lymphocytes in the mucosal layer, some of which exhibited intraepithelial invasion (arrows) (HE staining, ×100). ***B***: These lymphocytes had anisonucleosis and nuclear atypia (HE staining, ×400). These cells were positive for CD3 (***C***) (×400), CD56 (***D***) (×400), and perforin (***E***) (×400), and partially positive for granzyme B (***F***) (×400).

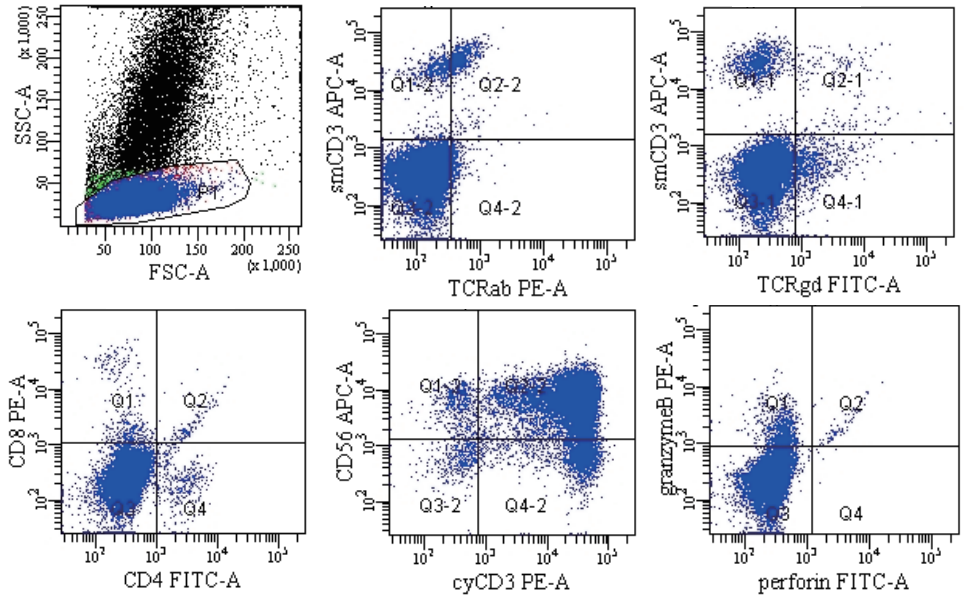

Fig. 4. Flow cytometric analysis of a biopsy specimen from the colonic lesion. CD3 was expressed in the cytoplasm (cy) but not on the surface membrane (sm). These lymphocytes were positive for CD56 and granzyme B (partially), but negative for CD4, CD8, smT-cell receptor (TCR)αβ, smTCRδγ, and cy-perforin.

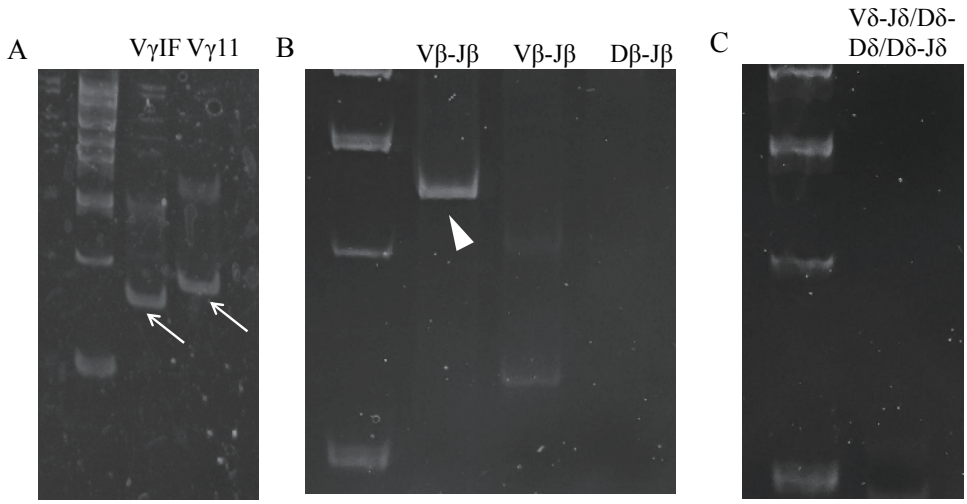

Fig. 5. PCR analysis of the mucosal lesion to assess TCR gene rearrangement. Monoclonal bands were seen in 2 lanes on TCR-γ gene analysis (***A***) (arrows). A monoclonal band on TCR-β (***B***) (arrowhead) but not TCR-δ (***C***) gene analysis was also noted.

stage 1 was made, classified in the high-intermediate group based on the International Prognostic Index.

After conservative management of the peritonitis, we treated him with a CHOP regimen (cyclophosphamide, doxorubicin, oncovin, and prednisolone). On colonofiberscopy, ulcerative lesions had improved; however, persistent dense proliferation of tumor cells was observed even in normal-looking mucosa on histopathological examination. We therefore switched the chemotherapy to a DeVIC (etoposide, ifosfamide, and carboplatin) regimen, but he developed progressive disease and died.

DISCUSSION

For the differential diagnosis of the present case, extranodal NK/ T-cell lymphoma, nasal type (ENKL), indolent T-cell lymphoproliferative disorder (LPD) of the gastrointestinal (GI) tract, and peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) were taken into consideration. In particular, the phenotype of lymphoma cells in the present case, cyCD3^{+}/CD56^{+}/smCD3^{-}/CD4^{-}/CD8^{-}, was identical to that of ENKL. However, we ruled out ENKL because of monoclonal rearrangement of the TCR-γ and TCR-β genes, negative EBER test, and colon-restricted disease, which is atypical for ENKL. Indolent T-cell LPD of the GI tract is usually negative for CD56. An exceptional case of T-cell LPD positive for CD56 was reported; however, the TCR gene rearrangement test was not performed.[7] The endoscopic findings and clinical course of our patient were also inconsistent with the clinical features of T-cell LPD. PTCL-NOS was excluded because of positive CD56, and negative smCD3, CD4, CD8, and smTCR-αβ and -γδ.

The cellular origin of MEITL and EATL was suggested to be transformed intestinal intraepithelial lymphocytes (IELs). IELs were found to exhibit diverse immunophenotypes depending on their differentiation pathway or resident site in a mouse model.[8] After positive selection in the thymus, some IELs differentiate into single positive T-cells and migrate into the intestine, whereas the remaining IELs are transferred to intestinal tissues when they are double-negative thymocytes and then differentiate into single CD8-positive T-cells after antigenic stimulation. Regarding the differentiation and migration of IELs, 60% of them in the small intestine are CD8-positive γδ T-cells and those in the large intestine are mainly αβ-type CD8-positive T-cells or CD4- and CD8-double-negative αβ- or γδ-type T-cells.

Regarding the cellular origin of the lymphoma cells in the present patient in relation to the above natural history of IELs, the involvement of double-negative thymocytes in the ontogenesis of T-cells was considered because the tumor cells expressed cyCD3 without smCD3, CD4, CD8, smTCR-αβ, or -γδ. This phenotype has not been reported in MEITL, and thus, this patient may be the first reported case of MEITL with a possible cell origin from this T-cell stage. CD3 and TCR form a complex and function together;[9,10] therefore, both CD3 and TCR can be detected on the cell surface membrane when CD3 is expressed there and vice versa. We attempted to stain cytoplasmic TCR proteins for the present patient, but an appropriate antibody for cytoplasmic staining was not available.

At present, only 2 cases of MEITL/EATL type II tumor cells exhibiting smCD3 expression by FCM analysis have been reported.[11,12] However, we previously encountered 3 cases of EATL type II with smCD3 expression by FCM analysis (unpublished data). Regarding CD8 and CD4 expression in this type of lymphoma, all cases analyzed with FCM were positive for CD8 but not CD4, except for the present case. On immunohistochemical analysis, however, a number of cases exhibited double negativity for CD8 and CD4,[4,13] suggesting cyCD3 expression.

Perforin positivity on immunohistochemistry in the present patient may be atypical for T-cells at the thymocyte stage. Regarding perforin gene expression, however, it has been reported that transcription occurs at the stage of double-negative

thymocytes and translated protein is detectable at the next stage of double-positive thymocytes.[14] We speculate that aberrant detection of perforin protein may occur at the stage of double-negative thymocytes because of altered gene action in the neoplastic state. Regarding the expression of perforin protein, lymphoma cells in the present patient were negative on FCM but positive on immunohistochemistry. The reason for this discrepancy was unclear. We confirmed the perforin positivity on FCM using the same antibody with NK-cells from a healthy subject and lymphocytes from a patient with large granular lymphocytosis. The antibody used in the present analysis may not have worked because of possible epitope alteration in lymphoma cells.

The CHOP regimen is commonly employed as the first-line treatment for MEITL; however, the 2-year survival rate is 28%, reflecting the poor prognosis associated with this disease using conventional chemotherapy.[15] Although no established combination chemotherapy is available, the SMILE regimen (dexamethasone, methotrexate, ifosfamide, L-asparaginase, and etoposide) and IVE/MTX (ifosfamide, vincristine, and etoposide/methotrexate) treatment followed by autologous stem cell transplantation has demonstrated favorable effects.[4,16] Novel agents developed for PTCL, such as forodesine hydrochloride,[17] pralatrexate,[18] and romidepsin,[19] may improve the prognosis of patients with MEITL in combination with chemotherapy.

In conclusion, we report the first case of colonal MEITL with a phenotype of cyCD3 and double negativity of CD4/CD8, suggesting a cellular origin of double-negative thymocytes. To establish the exact cellular origin of MEITL, phenotypic analysis of tumor cells by FCM and immunohistochemistry in a large number of MEITL cases is needed.

CONFLICT OF INTEREST

The authors declare no conflicts of interest for this study.

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