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ADULT T CELL LEUKEMIA PRESENTING AS MULTIPLE SUBMUCOSAL TUMORS OF THE INTESTINE: DETECTION OF HTLV-I DNA BY POLYMERASE CHAIN REACTION

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INDEXING WORDS

adult T cell leukemia; HTLV-I; intestine; polymerase chain reaction

SYNOPSIS

We discuss a 45-year-old man who presented with ileus due to multiple submucosal tumors of the small and large intestines. Emergency operation was performed and histological examination of the tumors revealed pleomorphic lymphoma cells with helper / inducer T-cell phenotype. Polymerase chain reaction (PCR) analysis using DNA extracted from the paraffin-embedded material showed that the intestinal, jejunal and femoral lymphoma samples contained human T-cell leukemia virus type-I (HTLV-I) DNA. Although no atypical lymphocytes were found in the peripheral blood, a diagnosis of adult T-cell leukemia was made. The PCR analysis of HTLV-I infection using paraffin-embedded materials seems to be useful when we encountered patients with T-cell lymphoma in the gastrointestinal tract.

INTRODUCTION

Malignant lymphoma in the gastrointestinal (GI) tract is usually derived from B-cells; T-cell lymphoma is relatively rare.[1] Predisposing factors to malignant lymphoma in this region are celiac disease (gluten-sensitive enteropathy),[13] chronic inflammatory bowel disease,[11] immunosuppression associated with organ transplantation, prior radiotherapy, ureterosigmoid anastomosis[10] and lymphoid hyperplasia.[2] Recently a high incidence of malignant lymphoma in the GI tract of patients infected with human immunodeficiency virus (HIV) has also been reported.[3] On the other hand, human T-cell leukemia virus type I (HTLV-I) is the first human

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retrovirus isolated and its etiological role in adult T-cell leukemia (ATL) is firmly established. [16] There are 2 reports of ATL involving the GI tract, but a genotypical analysis of HTLV-I infection was not performed in those patients. [9,15]

In our case, a patient presented with multiple submucosal tumors of the small and large intestines without atypical lymphocytes in the peripheral blood. Tumor cells were analyzed by immunohistochemistry and polymerase chain reaction (PCR) analysis using paraffin-embedded materials. Based on these analyses, the patient was diagnosed as having ATL.

REPORT OF A CASE

A 45-year-old man was admitted to our hospital with complaints of abdominal pain, vomitting and abdominal fullness. Emergency surgery was performed to relieve ileus. Macroscopically, 12 submucosal tumors, which were from 1cm to 7cm in the diameter, were found between the Treitz's ligaments and the descending colon. Submucosal tumors were resected from ileum, cecum, ascending colon and upper portion of the descending colon, and the jejunum 10 cm, 13 cm and 16 cm distant from the Treitz's ligaments. One jejunal lymph node was also removed. The specimens were fixed in 10% formalin and paraffin sections were stained with hematoxylin-eosin. The peripheral blood leukocyte count was 8040/μl with 16.5% lymphocytes. Morphologically, atypical lymphocytes were not observed. Examination of the lymphocyte subset revealed 2% of B-cell and 83% of T-cell (42.0%CD4+, 37.3%CD8+ and a CD4/CD8 ratio of 1.13). HTLV-I antibody in the serum was detected by enzyme immunoassay and confirmed by Western blot assays. Antibody to HIV was negative. Hypercalcemia was not present. Bone marrow aspirate was normal. Hepatosplenomegaly, skin disease and abnormal neurological findings were not observed. Chest X-ray and whole body computed tomographic scan were normal except for left femoral lymphadenopathy. This lymph node was biopsied. According to the clinical stage of Ann Arbor, this patient was stage IV, and he is being treated with combination chemotherapy consisting of cyclophosphamide, doxorubicin, cisplatin, vincristin, predonisolone and bleomycin, and is now asymptomatic.

MATERIALS AND METHODS

Immunohistochemistry

Immunohistochemical analysis was performed using the avidin-biotin complex method and the following antibodies, UCHL-1 for T-cells, L-26 for B-cells and OPD4 for helper / inducer T cells. [17] All antibodies were purchased from DAKOPATTS.

HTLV-I antibody assay

The serum was studied employing enzyme immunoassay using the New Eitest-ATL (Eisai,

Japan). More than cut off index 1.0 by this analysis was considered positive.[8] Confirmation by Western blot analysis using MT2 cell lysate antigen[7] was done.

DNA preparation

Two 5 μ m thick sections were cut from paraffin embedded intestinal, jejunal and femoral lymph node tissues. Paraffin tissues of normal intestinal portion at the margins of the tumor and intestinal tissue of HTLV-I seropositive another patient were also used. These were deparaffinized in two washes of xylene, rehydrated in 99% ethanol and vacuum dried. The sections were resuspended in 200 μ l of digestion buffer (100mM NaCl, 10mM Tris-Cl, 25mM EDTA, 0.5% SDS, pH 8.4) containing 200 μ g/ ml Proteinase K, and incubated for 3-5 days at 37℃. After digestion the Proteinase K was heat-inactivated at 95℃ for 8 minutes, and DNA was purified by organic extraction steps.[4] MT2 cell line, which was used as the positive control for HTLV-I, was washed in phosphate buffered saline (PBS) two times, resuspended in 500 μ l of digestion buffer (50mM KCl, 1.5mM $MgCl_2$, 10mM Tris-Cl, 0.5% Tween 20) containing 100 μ g/ml Proteinase K and incubated at 37℃ overnight. The Proteinase K was inactivated at 95℃ for 8 minutes, and the supernatant of the digested product was used for PCR.[5] DNA from 50 μ l peripheral blood of this patient was prepared by treatment with digestion buffer (10mM Tris-Cl, 1 mM EDTA) and Proteinase K. The Proteinase K was inactivated at 95℃ for 10 minutes.

Primers for PCR

The primers used for the HTLV-I PCR were the 19 and 20 base oligonucleotides: SK54: 5' -CTTCACAGTCTCTACTGTGC-3' (position of first nucleotide: 3365), and SK55: 5' -CGGCAGTTCTGTGACAGGG-3' (position of first nucleotide: 3465). These primers, derived from the pol region of the HTLV-I genome, are HTLV-I specific.[6] They were synthesized on a DNA synthesizer (MilliGen / Biosearch, USA).

Polymerase chain reaction

PCR was performed in a total volume of 50 μ l containing 1x PCR reaction buffer (Pharmacia, Uppsala, Sweden), 200 μ M of each deoxynucleotide, 1.25 U of Taq polymerase (Pharmacia), and 1 μ M of each primer. The reactions were performed in a Perkin-Elmer-Cetus (PEC, Norwalk, Conneticut, USA) thermal cycler, programmed for 35 cycles of denaturation at 95℃ for 1minute, followed by primer annealing at 55℃ for 1 minute and extension at 72℃ for 2 minutes (5 minutes in the last cycle). 10 μ l of the amplified product was run on a 2% agarose gel, stained with ethidium bromide and photographed under ultraviolet light. ϕ x 174 DNA / Hae III digest was used as molecular weight standard.

Controls for PCR

MT2 cell line was used as a positive control for HTLV-I DNA and a cervical carcinoma served as a negative control. Intestinal lymphoma samples were also amplified with human papilloma

virus 16 (HPV 16) specific primers. A solution containing PCR mix without template DNA was also amplified.

RESULTS

Light microscopic findings

The wall of the intestine, from the lamina propria mucosa to the serosa was infiltrated diffusely by lymphoma cells. These consisted of pleomorphic cells with convoluted nuclei, and were mostly medium to large in size (Fig.1). Focal lymphatic invasion was observed. In the jejunal and femoral lymph nodes, the normal architecture of the nodes was lost and proliferation of lymphoma cells similar to those in the intestine was observed.

Immunohistochemical findings

The immunohistochemical studies of the lymphoma cells of the intestine and lymph nodes showed positive staining for T-cell (UCHL-1) and helper / inducer (OPD4) antigens (Fig.2). B-cell marker L26 was negative.

HTLV-I antibody assay

By enzyme immunoassay analysis, cut off index of this patient was 21.7. By Western blot analysis, the serum showed antibodies to P19,24 and 28.

PCR findings

Two samples of the intestinal lymphoma, jejunal and femoral lymph nodes and the HTLV-I infected MT2 cell line showed amplification product of the expected size (119 base pairs (bp)) when amplified using primers specific for HTLV-I sequences. Peripheral blood cells, normal intestinal tissue at the margins of the tumor and intestinal tissue of HTLV-I seropositive another patient showed no amplification with primers specific for HTLV-I. Negative controls including cervical carcinoma and a reaction lacking template DNA yield no amplification products with the HTLV-I primers. No PCR product was seen when the intestinal samples were amplified with primers specific for HPV 16 (Fig.3).

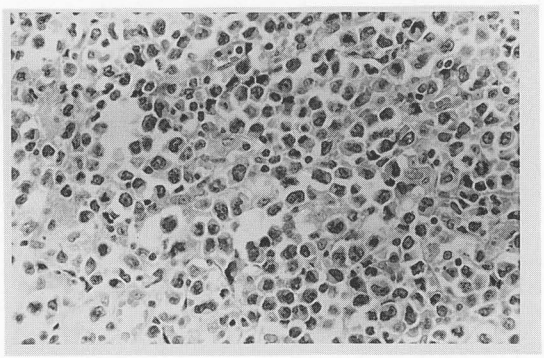

Fig.1. The lymphoma consists of pleomorphic cells with convoluted nuclei. H.E. x200

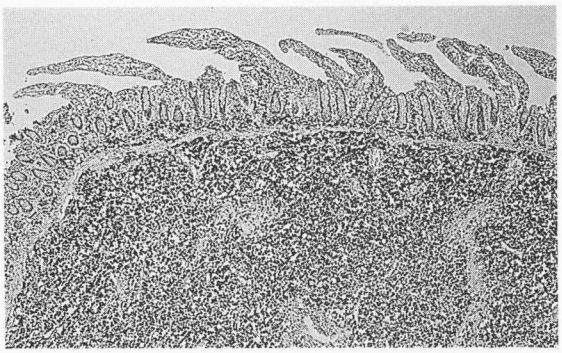

Fig.2. Lymphoma cells are positive for OPD4. x40

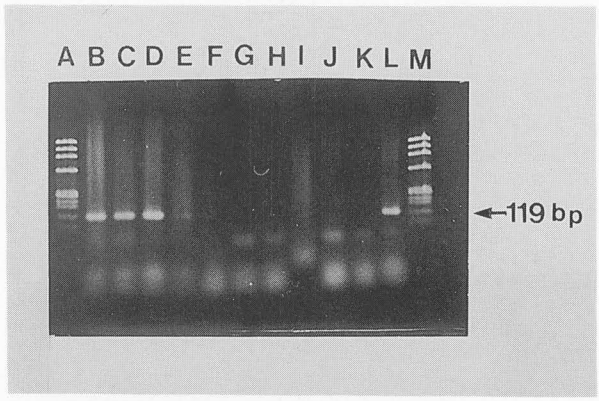

Fig.3 . Ethidium bromide stained agarose gel showing presence of PCR amplified DNA products of HTLV-I in intestinal samples, jejunal and femoral lymph nodes and MT2 cell line. Lane A and M: ϕ x 174 DNA / Hae III digest as a size marker. Lanes B and C: single band of amplification product of extracted DNA from intestinal samples of expected size (119bp). Lanes D and E: single band of amplification product of extracted DNA from (D) jejunal and (E) femoral lymph node samples of expected size (119bp). Lane F: amplification product of extracted DNA from peripheral mononuclear cells. Lane G: amplification product of extracted DNA from the margins of the intestinal tumor. Lane H: amplification product of extracted DNA from intestinal tissue of HTLV-I seropositive another patient. Lane I: amplification product of extracted DNA from intestinal samples primed with HPV16 primers. Lane J: amplification product of extracted DNA from cervical carcinoma samples containing HPV16 sequences primed with HTLV-I primers. Lane K: amplification product of PCR mix without template DNA. Lane L: amplification product of MT2 cell line primed with HTLV-I primers. The number on the right side indicate the size of HTLV-I amplification product in base pairs (bp).

DISCUSSION

ATL is a distinct clinical entity characterized by ①proliferation of ATL cells derived from mature helper T-cells and characterized by convoluted nuclei, ②frequent skin involvement, lymphadenopathy, and hepatosplenomegaly, ③a high leukemic cell count in the peripheral blood, ④hypercalcemia, and ⑤etiologic association of HTLV-I.[14] Sato *et al.*[9] observed GI tract involvement in 25% of autopsied ATL cases. In contrast, our case characterized by ileus due to intestinal submucosal tumors without the presence of circulating atypical lymphocytes, seems to be a rare variant of ATL. In fact, peripheral blood cells showed no amplification with primers specific for HTLV-I, and our blood samples harbored less than 0.1 viral copy / cell, which was the maximum sensitivity of the PCR. HTLV-I detection from the culture of peripheral blood mononuclear cells was not performed. The diagnosis of ATL in our case was based on the ① histology of the lymphoma showing diffuse pleomorphic lymphoid cells, ②the lymphoma cells in the GI tract and lymph nodes were helper / inducer T cells, ③antibody to HTLV-I was confirmed by enzyme immunoassay and Western blotting, ④the lymphoma cells in the intestine and lymph nodes contained HTLV-I DNA by PCR analysis.

ATL is uniformly associated with integration of HTLV-I proviral DNA in tumor cells. [16] The conventional proof of HTLV-I infection by genetic analysis requires a large amount of fresh material, and thus it is difficult to test for HTLV-I genomes in patients who are aleukemic and in whom frozen tissue is not saved at the time of surgery. PCR amplification of DNA sequences has made detection of HTLV-I possible on very small samples even from paraffin-embedded tissues. Using formalin-fixed, paraffin embedded tissue, Shibata *et al.* [12] have reported the detection of HTLV-I proviral sequences in lymphoma cells by PCR analysis. Using this analysis, we confirmed the integration of HTLV-I DNA in the intestinal lymphoma. Although T-cell lymphoma in the GI-tract is relatively rare, when encountered, we should test for HTLV-I sequences if morphologic and immunophenotypic data suggest the possibility of a diagnosis of ATL. In the next study, inverse PCR or linker-mediated PCR may be necessary for the investigation on monoclonal integration of HTLV-I genomes.

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