# Epstein-Barr Virus-Associated Peripheral T-cell Lymphoma with Gastrointestinal Tract Involvement

WINYOU MITARNUN, MD*,
JINTANA PRADUTKANCHANA, MSc*,
SATOMI TAKAO, MSc**,
VANNARAT SAECHAN, MSc*,
SUPAPORN SUWIWAT, MSc*,
TAKAFUMI ISHIDA, DSc**

**Abstract**

Peripheral T-cell lymphoma (PTCL) is a group of diseases which are common in Asia and areas of South and Central America. They are highly associated with the Epstein-Barr virus (EBV) infection. In the present study the authors evaluated patients with gastrointestinal involvement of PTCL with respect to clinical findings and outcome, pathologic features, and molecular analysis for EBV infection and the clonality of tumor cells. From January 1997 through December 2000, 7 patients with gastrointestinal tract involvement of PTCL were identified. The frequency of gastrointestinal tract involvement in the various types of PTCL was 5.4 per cent (7 of 129 cases). The pertinent clinical features were prolonged fever, weight loss, anemia, hepatosplenomegaly, lymphadenopathy, multiorgan involvement, and gastrointestinal bleeding. Laboratory results showed a significantly high serum level of alkaline phosphatase and lactate dehydrogenase, and abnormal coagulograms. Five patients died within 4 months after onset of illness, while two were in complete remission after chemotherapy. The tumor cell morphology was classified into three categories: small-sized cells, mixed medium- and large-sized cells, and large-sized cells. The antigenic phenotypes of the tumor cells were LCA+, CD3+, CD15-, CD16-, CD30-, CD45R0+, CD57-, CD68-, EMA-, βF1-, granzyme B+, TIA-1+, and p53+. The expression of CD4, CD8, CD56 and CD20 was variable. EBV-RNA expression by *in situ* hybridization (EBER-ISH) study was positive and T-cell receptor (TCR) beta and/or gamma gene rearrangements were detected in all patients. DNA sequence analysis showed high identity to the human TCR germline gene. PTCL with gastrointestinal tract involvement was associated with EBV infection. The tumor cells were mature T cells with some NK-cell antigenic expression and all demonstrated TCR gene rearrangements.

**Key word :** Peripheral T-cell Lymphoma, Epstein-Barr Virus, Gastrointestinal, T-cell Receptor Gene, Clonality

**MITARNUN W, SAECHAN V, PRADUTKANCHANA J, SUWIWAT S, TAKAO S, ISHIDA T**
**J Med Assoc Thai 2003; 86: 816-828**

* Department of Pathology, Faculty of Medicine, Prince of Songkla University, Songkhla 90110, Thailand.
** Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan.

Peripheral T-cell proliferative diseases/lymphomas are more commonly found in Asia than in Western countries[1-4]. They also have been reported with significant frequency in Central and South America[5-7]. This suggests a racial and/or environmental connection for the disease. Peripheral T-cell proliferative disease/lymphoma exhibits considerable heterogeneity in the clinical findings, morphologic pattern, immunology, and prognosis[4,8-11]. In 1993, the International Lymphoma Study Group proposed a classification for lymphoid neoplasms, entitled, A Revised European-American Classification of Lymphoid Neoplasms, (REAL classification), by using morphologic, immunologic, and genetic techniques[12, 13]. Recently, the World Health Organization (WHO) classification was proposed[14]. The mature NK/T-cell neoplasm classification in the proposed WHO scheme was largely adapted from the REAL classification. The WHO classification for predominantly extranodal malignant neoplasms was separated into four prototypes: extranodal NK/T-cell lymphoma, nasal type, enteropathy-type intestinal T-cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma, and hepatosplenic γδ T-cell lymphoma[15]. The involvement of peripheral T-cell lymphomas in the gastrointestinal tract may be found in three categories: enteropathy-type intestinal T-cell lymphoma, extranodal NK/T-cell lymphoma, nasal type, and gastrointestinal γδ T-cell lymphoma[15-17]. Of these, only the enteropathy-type intestinal T-cell lymphoma is considered to be a primary gastrointestinal tract T-cell lymphoma[14].

Gastrointestinal lymphoma accounts for 4 per cent to 20 per cent of all non-Hodgkin's lymphomas and is the most extranodal site of presentation[18]. The stomach is the major organ involved in gastrointestinal lymphoma. B-cell lymphoma of mucosal-associated lymphoid tissue (MALT) type is the commonest, and gastric MALT lymphoma may be associated with *Helicobactor pylori* and may undergo complete remission following eradication of *H. pylori* [19]. In Werstern countries, primary gastrointestinal lymphomas account for approximately 10 per cent to 15 per cent of non-Hodgkin's lymphoma cases, and the enteropathy-type intestinal T-cell lymphoma is the most common type[19,20].

Almost all gastrointestinal peripheral T-cell lymphoma cases which were reported from east Asia (Korea, Japan, China), Morocco, and Mexico were positive for Epstein-Barr virus (EBV) RNA by *in situ* hybridization study in most of the tumor cells[7,21-25]. Cases of gastrointestinal T-cell lymphoma which were reported from Western Europe and the United States of America showed only 16 per cent positive for EBV RNA in the tumor cells[7,26-31]. The majority of patients in the later series were cases with enteropathy-type intestinal T-cell lymphoma.

Enteropathy-type intestinal T-cell lymphoma is a specific peripheral T-cell lymphoma that occurs in association with celiac disease[27,32,33]. Celiac disease is relatively common in Europe. It occurs worldwide, but is quite rare in Asians and black Africans [33,34]. In one study, about 7 per cent of patients with celiac disease developed enteropathy-type intestinal T-cell lymphoma in a 25-year follow-up[27]. The enteropathy-type intestinal T-cell lymphoma, to our knowledge, has not been reported from Asia.

In the present study, the authors report 7 patients with gastrointestinal tract involvement of peripheral T-cell lymphoma. All cases were studied with respect to clinical findings and outcome, histopathologic and immunohistologic features, search for an association with EBV infection, and documentation of the clonality of the neoplastic T cells.

## MATERIAL AND METHOD

The prospective study was conducted in Songklanagarind University Hospital, Songkhla, Thailand, from January 1997 through December 2000 and follow-up continued until December 2001. There were 129 patients with various types of peripheral T-cell proliferative disease/lymphoma. Seven cases were found to have gastrointestinal tract lesions, and all of them were confirmed by histologic examination.

### Immunohistochemistry

Immunohistochemical stainings for leukocyte common antigen (LCA), CD3, CD4, CD8, CD15, CD16, CD20, CD30, CD45RO, CD56, CD57, CD68, T-cell receptor αβ (βF1), epithelial membrane antigen (EMA), granzyme B, T-cell intracellular antigen-1 (TIA-1), and p53 protein of tumor tissues were performed on formalin-fixed, paraffin embedded tissue using the following antibodies: monoclonal mouse anti-human LCA (CD45) (Zymed, CA, USA, 1 : 100), monoclonal mouse anti-human CD3 (Novocastra, UK, 1 : 120), monoclonal mouse anti-human CD4 (Novocastra, 1 : 25), monoclonal mouse anti-human CD8 (Dako, Denmark, 1 : 20), monoclonal rabbit anti-human CD15 (Zymed, 1 : 50), monoclonal mouse anti-human CD16 (Novocastra, 1 : 30), monoclonal mouse anti-human CD20 (Dako, 1 : 70), monoclonal mouse anti-

human CD 30 (Dako, 1 : 20), monoclonal mouse anti-human CD45RO (Zymed, 1 : 70), monoclonal mouse anti-human CD56 (Novocastra, 1 : 50); neuroblastoma was used as a positive control, monoclonal mouse anti-human CD57 (Novocastra, 1 : 50), monoclonal mouse anti-human CD68 (Dako, 1 : 100), monoclonal mouse anti-human βF1 (Endogen, MA, USA, 1 : 10), monoclonal mouse anti-human EMA (Dako, 1 : 20), monoclonal mouse anti-human granzyme B (Novocastra, 1 : 60), monoclonal mouse anti-human TIA-1 (Coulter, France, 1 : 30), p53 protein (Dako, 1 : 250), rabbit anti-mouse immunoglobulin (Dako, 1 : 200), swine anti-rabbit immunoglobulin (Dako 1 : 25), and horseradish peroxidase-mouse antiperoxidase (Dako, 1 : 100). The staining procedure was performed as previously described[35]. A tumor was considered p53 positive when more than 50 per cent of the tumor cell nuclei stained intensely with anti-p53.

### *In situ* hybridization

An in *situ* hybridization (ISH) study for the Epstein-Barr virus genomes was performed on formalin-fixed, paraffin embedded tissue using the fluorescein-conjugated EBV (EBER) oligonucleotides complementary to nuclear RNA portions of the EBER genes that were actively transcribed in latently infected cells. Briefly, tissue sections of 5 microns were deparaffinized with xylene, rehydrated in graded water-ethanol solutions, and digested with proteinase K (3 mg/L in 0.05 M Tris/HCl, pH 7.6) for 30 minutes at 37°C. After dehydration and air-drying, the fluorescein-conjugated (FITC) EBER oligonucleotide probes (Y 0017, Dako) were applied to the sections for 2 hours at 37°C. The following immunohistochemical detection system (K 046, Dako) was used: rabbit F(ab′) anti-FITC/AP for 30 minutes and a solution containing 5-bromo-4-chloro-3-indolyphosphate (BCIP) and nitro-blue tetrazolium (NBT) for 30-60 minutes. Then the slides were washed in running tap water and mounted in glycergel[36]. Appropriate positive and negative controls were run. ISH for EBV RNA was interpreted as positive when the positively stained tumor nuclei were more than 10 per cent of the tumor cell population. The amount of positive cells between 10-25 per cent was considered +1; 26-50 per cent, +2; 51-75 per cent, +3; and more than 75 per cent was considered +4.

### DNA extraction from tissue for PCR analysis

DNA extraction was performed using QIAamp DNA Mini Kit (QIAGEN, Germany). Three to five pieces of tissue section (3 to 5 microns) from paraffin blocks were taken, deparaffinized, resuspended in 180 μl of buffer ATL, digested with 50 μl of proteinase K, mixed by vortexing, and incubated at 55°C until the tissue was completely lysed. Further steps were followed as directed by the manufacturer's manual of tissue protocol[37]. The presence of DNA was confirmed by agarose-gel electrophoresis or by amplification of β–globin gene.

### Polymerase chain reaction (PCR) analysis

For the clonality study of the tumor cells, a T-cell receptor (TCR)-β chain and TCR-γ chain genes were used. Amplification of the TCR-β chain gene was carried out using the technique described by McCarthy et al[38]. The authors used three separate reactions with the following oligonucleotide primers combinations: D2+Jβ2.3, D2+Jβ2.6, and D2+Jβ2.7. For the monoclonal rearrangement of tumor cells, one or two clearly visible band/s with the expected size range (55 to 100 bp) were identified. Amplification of the TCR-γ chain gene was performed using the PCR technique as described by McCarthy et al[39,40]. The authors used two separate reactions with the following oligonucleotide primers combinations: VγI+VγIII/IV+Jγ1/2 (product sizes 70-95 bp), and VγII+Jγ1/2 (product sizes approximately 150-180 bp). For the monoclonal rearrangement, one or two cleary visible band/s were identified. DNA extracted from Raji and U8312 (a human T-cell lymphotropic virus type-1 positive cell line) were used as controls.

### DNA sequence analysis of TCR genes

The purified PCR products of TCR-β chain and TCR-γ chain genes were used as a template for automated sequencing analysis by using a ABI PRISM Dye Terminator Cycle Sequencing kit with AmpliTag DNA polymerase, FS (ABI PRISM™ Ready Reaction, Applied Biosystems Inc., CA, USA). The purified template (approximately 500 ng) was mixed with 8 μl of Terminator premix, 3.2 pmole of a sequencing primer and deionized water to a total volume of 20 μl. The mixture was overlaid with one drop of mineral oil and placed in the thermal cycler preheated to 96° C. Thermal cycling was performed on a Perkin-Elmer Cetus thermal cycler model 480 under the following conditions: 96°C denaturation for 15 sec, 60°C annealing for 15 sec and 60°C extension for 4 min with 25 total cycles. After the last cycle, the extension product was precipitated and redissolved in 4 μl of loading buffer containing 5 : 1 (vol/vol) of deionized forma-

mide with 50 mM EDTA, pH 8.0 and dextrane blue. The reaction tube was heated at 90°C for 2 min before loading onto a 6 per cent polyacrylamide sequencing gel for analysis in the Applied Biosystems Automated DNA sequencer, model 373A. The sequence obtained from each run was analysed with the assistance of SeqEd, a software program (Applied Biosystems Inc.) and compared with the sequence in Genbank using the Blast program.

## RESULTS

### Clinical features

Clinical data, pathologic findings, therapeutic interventions and outcomes of the patients are summarized in Table 1, and the pertinent laboratory findings are summarized in Table 2. Of the 129 patients with various types of peripheral T-cell proliferative disease/lymphoma, seven (5.4%) patients were found to have gastrointestinal tract lesions (4 males and 3 females, ranging in age from 23-71 years). All of these seven patients showed a prolonged fever which had lasted from one to six months. Five patients had hepatosplenomegaly; of these, four (case nos. 2, 3, 4, 5) were histologically confirmed to have peripheral T-cell lymphoma involving the liver. Three patients had peripheral T-cell lymphoma in the lymph nodes. Bone marrow involvement was found in three patients (case nos. 2, 4, 5). Case no. 7 had an extranodal NK/T-cell lymphoma, nasal type, in the nasopharynx two years before the gastrointestinal symptoms developed. Case no. 3 had peripheral T-cell lymphoma at the base of the tongue. An autopsy from case no. 5 revealed multiorgan involvement of peripheral T-cell lymphoma which included the liver, spleen, brain, and both adrenal glands.

The hemograms revealed anemia in all cases. The number of white blood cells and platelets varied. Most of these patients had high serum levels of alkaline phosphatase and lactate dehydrogenase, and abnormal prolonged coagulograms.

Of three patients who received multidrug chemotherapy, two (case nos. 2, 6) achieved a complete remission in the 46-month and 42-month follow-up; and one of them (case no. 7) died. In four patients who did not have chemotherapy, the disease followed a rapidly progressive course and all of them died within one to four months after the first onset of illness.

### Pathological features

#### *Gross pathology*

The major tumor sites in the gastrointestinal tract were the ileocaecal region and colon. Lesions in the stomach, duodenum, and jejunum were also found in some cases. In most cases, multicentric lesions involving different areas of infiltrative and ulcerative lesions were the major feature. However, two patients (case nos. 1, 6) had a single lesion. Perforation of the bowel was seen in cases 1 and 3.

#### *Histopathology*

There was a wide spectrum of cytologic morphology of tumor cells ranging from small-sized to large atypical and hyperchromatic cells. There was also an admixture of these types of cells. Some may be difficult to distinguish from an inflammatory process. Infiltrations with eosinophils, monocytes, and plasma cells were variable. The authors classified the tumor morphology into three categories: small-sized cells, mixed medium- and large-sized cells, and large-sized cells.

Small-sized cells (Fig. 1): the infiltrate was composed of small round lymphocytes, but there was no marginal zone pattern. Necrosis or angiocentric pattern were rarely observed. Infiltration with eosinophils and plasma cells varied from mild to moderate. The lesions were confined mainly to the mucosa and upper part of the muscular wall.

Mixed medium- and large-sized cells (Fig. 2): the infiltrate was composed of medium-sized cells, large atypical hyperchromatic cells, and a few small-sized cells. An angiocentric pattern and coagulative necrosis were prominent features. Infiltration with eosinophils, monocytes and plasma cells varied from mild to massive. The lesions were located mainly in the mucosa and occasionally extended to the muscular wall and adventitia.

Large-sized cells (Fig. 3): The infiltrate was mostly composed of large-size cells. The cells were pleomorphic, and had hyperchromatic nuclei and abundant mitoses. An angiocentric pattern and coagulative necrosis were very prominent features. Apoptotic cells were seen in large numbers. Infiltration of monocytes with massive hemophagocytosis was observed in the tumor areas, and in the lumen of nearby vessels. Infiltration with eosinophils and plasma varied from minimal to absent.

**Table 1. Clinical and histologic findings.**

| Patient no. | Age (yr)/ sex | Presenting symptom and sign | Operation site | Gross and size | Histologic type | Histologic finding in other site | Post-operative therapy | Follow-up/ outcome |
|---|---|---|---|---|---|---|---|---|
| 1 | 28/M | High-grade fever 2 mos, hepatosplenomegaly, lymphadenopathy, ascites, bowel perforation | Terminal ileum and right half colon resection | Bulky tumor at ileum and caecum 3 x 4 x 8 cm, caecal perforation | SC | PTCL in lymph node | NA | DOD, 4 mos |
| 2 | 23/F | Low-grade fever and bloody diarrhea 1 mo, hepatosplenomegaly | Duodenum and ileum biopsy | Edema of small bowel mucosa | SC | PTCL in liver<br>RH in lymph node | CHOP | CR, 46 mos |
| 3 | 71/F | High-grade fever 1 mo, weight loss 10 kg, bloody diarrhea, hepatosplenomegaly | Terminal ileum and right half colon resection | Multiple ulcers at ileum upto 5 x 2 cm, ileal perforation | SC | PTCL in liver<br>AILD in lymph node<br>PTCL of the tongue | NA | DOD, 2 mos |
| 4 | 28/M | High-grade fever 1 mo, bloody diarrhea 1 week, hepatosplenomegaly | Jejunum, ileum and right half colon resection | Multiple infiltrative lesions and ulcers (up to 3 cm) in jejunum, ileum and colon | MLC | PTCL in liver | NA | DOD, 2 mos |
| 5 | 27/M | High-grade fever 1 mo, weight loss 5 kg, bloody diarrhea, hepatosplenomegaly | Terminal ileum and total colon resection | Multiple small infiltrative lesions and ulcers in entire colon | MLC | Autopsy showed PTCL in stomach and small bowel, liver lymph nodes, spleen, bone marrow, brain and adrenal glands | NA | DOD, 1 mo |
| 6 | 34/F | Fever and abdominal pain 6 mos, weight loss 7 kg | Duodenum and portion of jejunum resction | An ulcer at duodenum, 5 x 3 cm | LC | - | CHOP | CR, 42 mos |
| 7 | 51/M | High-grade fever 1 mo, maculopapular rash and skin ulcers on body and face, status post radiation therapy for NK/T in nasopharynx 2 yrs ago, bloody diarrhea 1 day | Left half colectomy, biopsy from duodenum and jejunum | Multiple small infiltrative lesions and ulcers (0.7-1.2 cm) in colon | LC | Cutaneous T-cell lymphoma<br>RH in lymph node | Vincristin, Endoxan | DOD, 2 mos |

Abbreviations :DOD = dead of disease, CR = complete remission after chemotherapy, NA = not applicable, SC = small-sized cell, MLC = mixed medium- and large-sized cells, LC = large-sized cell, PTCL = peripheral T-cell lymphoma, RH = reactive hyperplasia, AILD = angioimmunoblastic T-cell lymphoma, NK/T = extranodal NK/T-cell lymphoma, nasal type.

**Table 2. Laboratory findings.**

| Test | Case no. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Hemoglobin, g/L | 85 | 82 | 83 | 103 | 81 | 81 | 88 |
| White blood cell count, $x10^9$/L | 2.5 | 24.6 | 1.2 | 9.5 | 5.7 | 4.7 | 10.5 |
| Platelets, $x10^9$/L | 167 | 574 | 262 | 26 | 66 | 305 | 330 |
| Total protein, g/L | 45 | 48 | 50 | 30 | 46 | 61 | 57 |
| Albumin, g/L | 21 | 24 | 29 | 19 | 13 | 30 | 33 |
| Total bilirubin, μmol/L | 23.8 | 8.5 | 22.1 | 251.7 | 132.6 | 6.8 | 10.8 |
| Conjugated bilirubin, μmol/L | 19.2 | 3.4 | 13.7 | 161.5 | 107.2 | 5.4 | 3.4 |
| Aspartate aminotransferase, U/L (0-35) | 68 | 46 | 54 | 1970 | 15 | 15 | 38 |
| Alanine aminotransferase, U/L (0-35) | 27 | 43 | 27 | 161 | 14 | 9 | 35 |
| Alkaline phosphatase, U/L (30-120) | 287 | 218 | 235 | 266 | 1003 | 80 | 55 |
| Lactate dehydrogenase, U/L (230-460) | 512 | 280 | 504 | ND | 356 | 491 | ND |
| Activated PTT, s (28-31) | 69 | 34 | ND | 64.6 | 54 | 26.5 | 37.6 |
| Prothrombin time, s (13-15) | 25.5 | 13 | ND | 21.3 | 38.5 | 13.3 | 16.7 |
| Anti HIV (by ELISA) | ND | neg | neg | neg | neg | neg | neg |

Abbreviations : ND = not done, neg = negative, PTT = partial thromboplastin time.

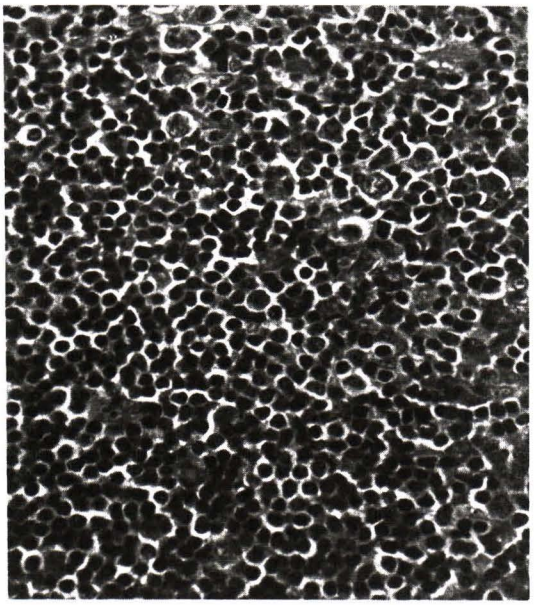

**Fig. 1. Small-sized tumor cells. The majority of tumor cells are small with round to only slightly irregular nuclei and have a homogeneous pattern.**

**Fig. 2. Mixed medium- and large-sized tumor cells. The tumor cells are medium-sized and large-sized cells with atypical hyperchromatic nuclei. Note the angiocentric pattern.**

Cases 1, 2 and 3 were classified as small-sized cells, cases 4 and 5 as mixed medium- and large-sized cells, and cases 6 and 7 as large-sized cells. In the group of small-sized cells, none showed an angiocentric pattern and only case 1 showed coagulative necrosis. An angiocentric pattern and coagulative necrosis were found in all patients with the histologic types of mixed medium- and large-sized cells, and

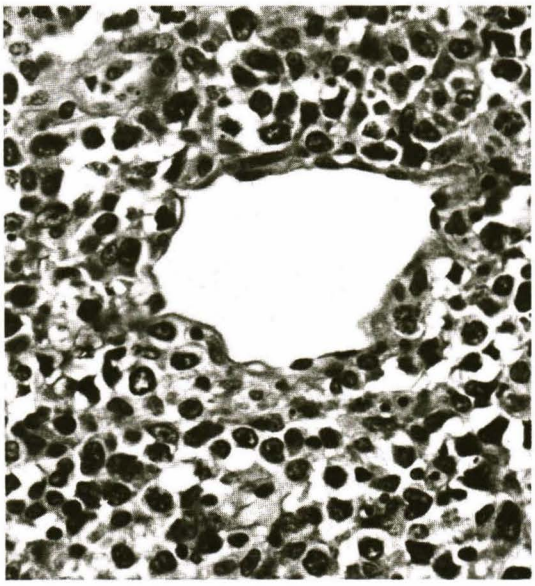

**Fig. 3. Large-sized tumor cells. Angiocentric pattern is a prominent feature. The tumor cells are pleomorphic, and have hyperchromatic nuclei and abundant mitoses.**

large-sized cells. The adjacent mucosal tissue and/or the uninvolved small bowel mucosa in all cases revealed no evidence of celiac disease.

### *Immunohistohemistry*

Results of the immunohistochemistry are summarized in Table 3. The phenotypes of tumor cells in these cases were LCA+, CD3+ (membrane pattern), CD15-, CD16-, CD30-, CD45RO+, CD57-, CD68-, βF1-, EMA-, granzyme B+, TIA-1+, and p53+. The expression of CD4, CD8, CD56 and CD20 was variable. One case expressed CD4, three cases expressed CD8, and one case expressed CD56. Case no. 4 expressed both CD20 and CD3, which are the markers of B cell and T cell, respectively.

### *In situ hybridization*

The presence of EBV RNA (EBER) in tumor cells was detected in all cases, ranging from +2 to +4 (Table 3). Tumors of the mixed medium- and large-sized cells, and the large-sized cells morphologies, showed a strongly positive reaction.

### *Clonality study*

Results of the clonality studies of these tumor cells are shown in Fig. 4 and 5. By using three sets of

**Table 3. Immunophenotypes and EBV RNA (EBER) of tumor cells in 7 patients.**

| Case no. | Phenotypes | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LCA | CD3 | CD4 | CD8 | CD15 | CD16 | CD20 | CD30 | CD45RO | CD56 | CD57 | CD68 | βF1 | EMA | GrB | TIA-1 | p53 | EBV RNA |
| 1 | + | + | - | - | - | - | - | - | + | - | - | - | - | - | + | + | + | +2 |
| 2 | + | + | ND | - | - | ND | - | - | + | ND | - | - | ND | ND | ND | ND | ND | +2 |
| 3 | + | + | - | + | - | - | - | - | + | - | - | - | - | - | + | + | + | +3 |
| 4 | + | + | + | + | - | - | + | - | + | + | - | - | - | - | + | + | + | +4 |
| 5 | + | + | - | + | - | - | - | - | + | - | - | - | - | - | + | + | + | +4 |
| 6 | + | + | - | - | - | - | - | - | + | - | - | - | - | - | + | + | + | +4 |
| 7 | + | + | - | - | - | - | - | - | + | - | - | - | - | - | + | + | + | +4 |

Abbreviations : LCA = leukocyte common antigen, GrB = granzyme B, ND = not done.

oligonucleotide primers for TCR-β gene, the monoclonal band was detected in four cases; case nos. 1, 2, 3 and 6 (Fig. 4). For the TCR-γ gene, the monoclonal band/s were detected in four cases; case nos. 4, 5, 6 and 7 (Fig. 5).

### *TCR sequence analysis*

Results of the TCR gene- sequence analysis (sense strand in case nos. 1, 2, 3 and anti-sense strand in case nos. 4, 5, 6, 7) are summarized in Table 4. All of these cases confirmed the monoclonality. PCR products had a very high identity to the human TCR germline gene.

## DISCUSSION

Peripheral T-cell proliferative disease/lymphoma has been recognized as a common group of diseases in Southern Thailand[4,41-43]. In our institute, a 700-bed hospital, the authors found 129 cases in a 4-year prospective study. Of these, 7 had gastrointestinal tract involvement. The patients presented with prolonged fever, weight loss, anemia, hepatosplenomegaly, abnormal prolonged coagulograms, and significantly high serum levels of alkaline phosphatase and lactate dehydrogenase. All four patients with liver involvement of peripheral T-cell lymphoma showed high serum levels of alkaline phosphatase. The pertinent gastrointestinal symptom was bloody diarrhea, and some patients had bowel perforation.

Gastrointestinal non-Hodgkin's lymphoma accounts for 4 per cent to 20 per cent of all non-Hodgkin's lymphoma cases, and is the most common extranodal site of presentation[18]. Intestinal B-cell lymphomas appear as polypoid masses or annular lesion in the distal and terminal ileum[20]. Enteropathy-type intestinal T-cell lymphomas in Western countries are mainly found in the proximal small intestine, in contrast to intestinal T-cell lymphomas in Asia and in the presented study, which tended to be located in the terminal ileum and the large intestine[20,21].

Extranodal peripheral T-cells and NK-cell neoplasms are classified into 4 major categories: nasal, intestinal, subcutaneous panniculitis-like, and hepatosplenic γδ[15]. EBV-positive extranodal NK/T-cell lymphoma, nasal type, which frequently involves the intestine as a secondary site, is almost always CD56+ (70% to 100%), CD3 (membrane)-, CD3 (cytoplasm)+,

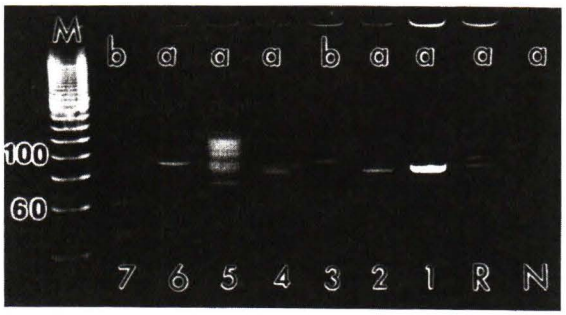


**Fig. 4. PCR T-cell receptor-beta gene amplification using three pairs of primers: D2+Jβ2.3 (a), D2+Jβ2.6 (b), and D2+Jβ2.7 (c). Lanes 1 through 7 are cases 1 to 7 respectively. Lane R is Raji control, Lane N is negative control, M is ladder markers. The monoclonal band is detected in cases 1, 2, 3, and 6, (10% acrylamide gel).**

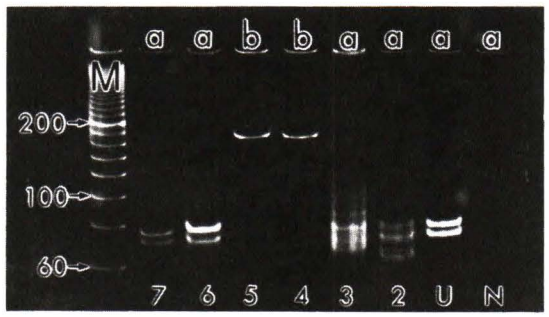


**Fig. 5. PCR T-cell receptor-gamma gene amplification using two sets of primers: Vγ1+VγIII/IV+Jγ1/2 (a), and VγII+Jγ1/2 (b). Lanes 2 through 7 are cases 2 to 7 respectively. Lane U is U8312 positive control, Lane N is negative control, M is ladder markers. The monoclonal band is detected in cases 4, 5, 6, and 7, (case no. 1 not amplified, 10% acrylamide gel).**

**Table 4. Results of TCR gene-sequence analysis.**

| Case | Primers | PCR product (bp) | Part of sequencing product (bp) | Homology site of TCR germline gene | Identity bp | % |
|---|---|---|---|---|---|---|
| 1 | Vγ1 + VγIII/IV + Jγ 1/2 | 70-95 | 43 | gi/5566238/gb/AF 159056.1 (Vγ1 regions) | 34/34 | 100 |
| 2 | D2 + Jβ2.3 | 80-90 | 59 | gi/1197256/emb/x86129.1 (71-116) | 46/46 | 100 |
| 3 | D2 + Jβ2.6 | 80-90 | 68 | gi/338852/gb/M 14159.1 (2099-2148) | 46/50 | 92 |
| 4 | Vγ1I + Jγ 1/2 | 160-180 | 140 | gi/5566238/gb/AF 159056.1 (56793-56911) | 117/119 | 98 |
| 5 | Vγ1I + Jγ 1/2 | 160-180 | 141 | gi/5566238/gb/AF 159056.1 (56793-56924) | 131/132 | 99 |
| 6 (upper band) | Vγ1 + VγIII/IV + Jγ 1/2 | 80-90 | 62 | gi/3047019/gb/AF 057177.1 (Vγ1 regions) | 30/30 | 100 |
| 6 (lower band) | Vγ1 + VγIII/IV + Jγ 1/2 | 70-80 | 53 | gi/3047019/gb/AF 057177.1 (Vγ1 regions) | 29/29 | 100 |
| 7 | Vγ1 + VγIII/IV + Jγ 1/2 | 70-95 | 43 | gi/5566238/gb/AF 159056.1 (Vγ1 regions) | 19/19 | 100 |

high prevalence of p53 overexpression, and lacks features associated with the clonal rearragement of TCR-β and TCR-γ. CD16 expression varies from 10 per cent to 60 per cent[15,44-50]. Based on the morphology, the disease is characterized by a broad cytologic spectrum, ranging from cytologically benign, which cannot be distinguished from an inflammatory process, to the definite malignant morphology[16]. An angiocentric pattern on histologic examination is a common feature, and necrosis is seen in most cases[15].

In gastrointestinal tract involvement of peripheral T-cell lymohomas, granzyme B-positive cells are mainly associated with enteropathy-type intestinal T-cell lymphoma. A total of 77 per cent of enteropathy-type intestinal T-cell lymphoma cases *versus* 14 per cent of the non-enteropathy-type intestinal T-cell lymphoma cases are granzyme B positive in the tumor cells[49]. Although all the six cases studied were positive for granzyme B and TIA-1, the clinical history and the adjacent mucosal tissue and/or the uninvolved small intestine nucosa in all cases failed to reveal evidence of celiac disease, which is the preceding lesion of the enteropathy-type intestinal T-cell lymphoma[32,33].

The antigenic phenotype of this reported series showed T-cell markers with some NK-cell antigenic expression. The NK-cell-associated marker CD56, which has been considered as a universally positive marker in the extranodal NK/T-cell lymphoma, nasal type, was negative in 5 of 6 cases in our series. The neuroblastoma positive control and the internal positive control (neurons in tissue section) gave a strongly positive reaction. The tumor cells showed no expression of CD16, whereas the activated macrophages which infiltrated in the tumor area showed intense membrane staining. The intracellular cytotoxic molecules (granzyme B, TIA-1), and the p53 expression, were detected in all tested cases. In the present study, the cases were all positive for the EBV genome, similar to previous reports of extranodal NK/T-cell lymphoma, nasal type, but there was evidence against the diagnosis in that they all demonstrated T-cell receptor gene rearrangements, and a majority of cases failed to express CD56. The findings suggest that these cases were not extranodal NK/T-cell lymphoma, nasal type, eventhough case no.1 had a preceding extranodal NK/T-cell lymphoma, nasal type, in the nasopharynx. Another important finding was the expression of CD20 in case no. 4. This concurred with our previous report on a high-grade hepatic peri-

pheral T-cell lymphoma which showed expression of both CD45R0 and CD20 in 3 of 8 cases[4], and a recent case report of peripheral T-cell lymphoma with aberrant expression of CD20 and CD79a[51].

All of the presented seven cases demonstrated either TCR-β or TCR-γ gene rearrangements. Cases 2 and 3 had only the rearrangement of the TCR-β locus, and cases 4, 5 and 7 had only the rearrangement of the TCR-γ locus. This finding was slightly different from a previous report of peripheral T-cell lymphomas where the TCR-β locus was found to be rearranged in 78 per cent of cases *versus* 96 per cent of the TCR-γ locus[52]. The TCR sequence analysis in all cases confirmed the monoclonality and had a high identity to the human TCR germline gene. This indicated the neoplastic nature instead of an inflammatory process of peripheral (mature) T cell in the presented cases.

## ACKNOWLEDGEMENTS

This study was supported by the Thailand Research Fund (TRF), grant no. BRG/7/2542, and was supported in part by the Core University System Exchange Programme under the Japan Society for the Promotion of Science (JSPS), coordinated by the University of Tokyo Graduate School of Medicine, Japan, and Prince of Songkla University, Faculty of Medicine, Thailand.

(Received for publication on October 7, 2002)

## REFERENCES


1. Lukes RJ, Taylor CR, Parker JW, Lincole TL, Pattengale PK, Tindle BH. The morphologic and immunologic surface marker study of 229 cases of non-Hodgkin's lymphomas and related leukemia. Am J Pathol 1978; 90: 461-86.
2. Kadin ME, Berard CW, Namba K, Wakasa H. Lymphoproliferative diseases in Japan and western countries. Human Pathol 1983; 14: 745-72.
3. Aozasa K, Ohsawa M, Tajima K, et al. Nationwide study of lethal midline granuloma in Japan: Frequencies of Wegener granulomatosis, polymorphic reticulosis, malignant lymphoma and other related conditions. Int J Cancer 1989; 44: 63-6.
4. Mitarnun W, Kietthubthew S, Suwiwat S. Hepatic peripheral T-cell lymphoma: A spectrum of liver pathology and clinical correlation. J Med Assoc Thai 1997; 80: 219-32.
5. Arber DA, Weiss LM, Albujar PF, Chen YY, Jaffe ES. Nasal lymphoma in Peru, high incidence of T-cell immunophenotype and Epstein-Barr virus infection. Am J Surg Pathol 1993; 17: 392-9.
6. Navarro-Roman L, Zarate-Osorno A, Meneses A, Kingma DW, Jaffe ES. High grade AIL and Epstein-Barr virus infection in 22 cases from Mexico. Mod Pathol 1994; 7: 117A.
7. Quintanilla-Martinez L, Lome-Maldonado C, Ott G, et al. Primary non-Hodgkin's lymphoma of the intestine: High prevalence of Epstein-Barr virus in Mexican lymphomas as compared with European cases. Blood 1997; 89: 644-51.
8. Pinkus GS, O'Hara CJ, Said JW. Peripheral/post-thymic T-cell lymphomas: A spectrum of disease. Clinical, pathologic, and immunologic features of 78 cases. Cancer 1990; 65: 971-98.
9. Weiss LM, Crabtree GS, Rouse RV, Warnke R. Morphologic and immunologic characterization of 50 peripheral T-cell lymphomas. Am J Pathol 1985; 118: 316-24.
10. Weisenburger DD, Astorino RN, Glassy FJ, Miller CH, Mackenzie MR, Caggiano V. Peripheral T cell lymphoma. A clinicopathologic study of a morphologically diverse entity. Cancer 1985; 56: 2061-8.
11. Chott A, Augustin I, Wrba F, et al. Peripheral T-cell lymphomas: A clinicopathologic study of 75 cases. Hum Pathol 1990; 21: 1117-25.
12. Harris NE, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: A proposal from international lymphoma study group. Blood 1994; 84: 1361-92.
13. Chan JKC, Banks PM, Cleary ML, et al. A revised European-American classification of lymphoid neoplasms proposed by the international lymphoma study group. A summary version. Am J Clin Pathol 1995; 103: 543-60.
14. Jaffe ES, Harris NL, Debold J, Muller-Hermelink HK. World Health Organization classification of neoplastic diseases of hematopoietic and lymphoid tissues. A progress report. Am J Clin Pathol 1999; 111 (Suppl 1): S8-12.
15. Jaffe ES, Krenacs L, Kumar S, Kingma DW, Raffeld M. Extranodal peripheral T-cell and NK-cell neoplasms. Am J Clin Pathol 1999; 111 (Suppl 1): S46-55.
16. Jaffe ES, Chan JKC, Su IJ, et al. Report of workshop on nasal and related extranodal angiocentric T/natural killer cell lymphomas. Definitions, differential diagnosis, and epidemiology. Am J Surg Pathol 1996; 20: 103-11.
17. de Wolf-Peeters C, Achten R. γδ T-cell lymphoma: A homogenous entity? Histopathol 2000; 36: 294-305.
18. d' Amore F, Christensen BE, Bricker H, et al. Clinicopathological features and prognostic factors in extranodal non-Hodgkin's lymphomas. Eur J Cancer 1991; 27: 1201-8.
19. Isaacson PG. Gastrointestinal lymphomas of T- and B-cell types. Mod Pathol 1991; 12: 151-8.
20. Crump M, Gospodarowicz M, Shepard FA. Lymphoma of the gastrointestinal tract. Semin Oncol 1999; 26: 324-37.
21. Huh J, Cho K, Heo DS, Kim JE, Kim CW. Detection of Epstein-Barr virus in Korean peripheral T-cell lymphoma. Am J Hematol 1999; 60: 205-14.
22. Lee SS, Jang JJ, Cho KJ, Khang SK, Kim CW. Epstein-Barr virus-associated primary gastrointestinal lymphoma in non-immunocompromised patients in Korea. Histopathol 1997; 30: 234-42.
23. Chan JKC, Sin VC, Wong KF, et al. Nonnasal lymphoma expressing the natural killer cell marker CD56: A clinicopathologic study of 49 cases of an uncommon aggressive neoplasm. Blood 1997; 89: 4501-13.
24. Abe Y, Muta K, Ohshima K, et al. Cytotoxic T-cell lymphoma diffusely involving the entire gastrointestinal tract associated with Epstein-Barr virus and tubercle bacilli infection. Int J Hematol 2000; 71: 379-84.
25. Lavergne A, Brocheriou I, Delfau MH, Copie-Bergman C, Houdart R, Gaulard PH. Primary intestinal gamma-delta T-cell lymphoma with evidence of Epstein-Barr virus. Histopathol 1998; 32: 271-6.
26. de Bruin PC, Jiwa NM, Oudejans JJ, Radaszkiewicz T, Meijer CJLM. Epstein-Barr virus in primary gastrointestinal T-cell lymphoma. Association with gluten-sensitive enteropathy, pathologic features, and immunophenotype. Am J Pathol 1995; 146: 861-7.
27. Ilyas M, Niedobitek G, Agathanggelou A, et al. Non-Hodgkin's lymphoma, coeliac disease, and Epstein-Barr virus: A study of 13 cases of entero-

pathy-associated T-and B- cell lymphoma. J Pathol 1995; 177: 115-22.
28. de Bruin PC, Connolly CE, Oudejans JJ, et al. Enteropathy-associated T-cell lymphomas have a cytotoxic T-cell phenotype. Histopathol 1997; 31: 313-7.
29. Arnulf B, Copie-Bergman C, Delfau-Larue MH, et al. Non hepatosplemic γδ T-cell lymphoma: A subset of cytotoxic lymphomas with mucosal or skin location. Blood 1998; 91: 1723-31.
30. Ott MM, Ott G, Klinker H, Trunk MJF, Katzenberger T, Muller-Hermelink HK. Abdominal T-cell non-Hodgkin's lymphoma of the gamma/delta type in a patient with selective immunoglobulin A deficiency. Am J Surg Pathol 1998; 22: 500-6.
31. Ranheim EA, Jones C, Zehnder JL, Warnke R, Yuem A. Spontaneus relapsing clonal, mucosal cytotoxic T-cell lymphoproliferative disorder. Case report and review of the literature. Am J Surg Pathol 2000; 24: 296-301.
32. Holmes GKT, Prior P, Lane MR, Pope D, Allan RN. Malignancy in coeliac disease-effect of a gluten free diet. Gut 1989; 30: 333-8.
33. Gale J, Simmonds PD, Mead GM, Sweetenham JW, Wright DH. Enteropathy-type intestinal T-cell lymphoma: Clinical features and treatment of 31 patients in a single center. J Clin Oncol 2000; 18: 795-803.
34. Kagnoff MF. Celiac disease. Gastroenterol Clin N Am 1993; 21: 405-25.
35. Boenisch T. Staining methods. In: Naish SJ, eds. Handbook of immunochemical staining methods. Capinteria, Calif: Dako Corp; 1989: 13-8.
36. Mitarnun W, Saechan V, Pradutkanchana J, et al. Epstein-Barr virus-associated non-Hodgkin's lymphoma of B-cell origin, Hodgkin's disease, acute leukemic, and systemic lupus erythematosus: A serologic and molecular analysis. J Med Assoc Thai 2002; 85: 552-9.
37. QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook. QIAGEN, Germany 1999: 28-31.
38. McCarthy KP, Sloane JP, Kabarowski JHS, Matutes E, Wiedemann LM. The rapid detection of clonal T-cell proliferations in patients with lymphoid disorders. Am J Pathol 1991; 138: 821-8.
39. McCarthy KP, Sloane JP, Kabarowski JHS, Matutes E, Wiedemann LM. A simplified method detecting of clonal rearragements of the T-cell receptor-γ chain gene. Diagn Mol Pathol 1992; 1: 173-9.
40. Diss TC, Watts M, Pan LX, Burke M, Linch D, Isaacson PG. The polymerase chain reaction in the demonstration of monoclonality in T cell lymphomas. J Clin Pathol 1995; 48: 1045-50.
41. Mitarnun W, Suwiwat S, Pradutkanchana J, et al. Epstein-Barr virus-associated peripheral T-cell and NK-cell proliferative disease/lymphoma: A clinicopathologic, serologic and molecular analysis. Am J Hematol 2002; 70: 31-8.
42. Mitarnun W, Kietthubthew S, Suwiwat S. Intrahepatocellular erythrocyte inclusions with hepatic sinusoidal infiltrates and splenomegaly. Arch Pathol Lab Med 1990; 114: 148-54.
43. Mitarnun W. Granulomatous reaction in peripheral T-cell proliferative disease: A case report. J Med Assoc Thai 1997; 80: 795-8.
44. Nakamura S, Suchi T, Koshikawa T, et al. Clinicopathologic study of CD56 (NCAM) positive angiocentric lymohoma occuring in sites other than upper and lower respiratory tract. Am J Surg Pathol 1995; 19: 284-96.
45. Gorp JV, Weiping L, Jacobse K, et al. Epstein-Barr virus in nasal T-cell lymphomas (polymorphic reticulosis/midline malignant reticulosis) in western China. J Pathol 1994; 173: 81-7.
46. Ng CS, Chan JKC, Lo STH. Expression of natural killer cell markers in non-Hodgkin's lymphomas. Hum Pathol 1987; 18: 1257-62.
47. Kanavaros P. Lescs MC. Briere J, et al. Nasal T-cell lymphoma: A clinicopathologic entity associated with peculiar phenotype and with Epstein-Barr virus. Blood 1993: 81: 2688-95.
48. de Bruin PC, Kummer JA, van de Valk P, et al. Granzyme B-expressing peripheral T-cell lymphoma: Neoplastic equivalents of activated cytotoxic T-cell with preference for mucosa-associated lymphoid tissue localization. Blood 1994; 84: 3785-91.
49. Gaal K, Sun NCJ, Hernandez AM, Arber DA. Sinonasal NK/T-cell lymphomas in United States. Am J Surg Pathol 2000; 24: 1511-7.
50. Quintanilla-Martinez L, Franklin JL, Querrero I, et al. Histological and immunophenotypic profile of nasal NK/T cell lymphoma from Peru: High prevalence of p53 overexpression. Hum Pathol 1999; 30: 849-55.
51. Yao X, Teruya-Feldstein J, Raffeld M, Sorbara L, Jaffe ES. Peripheral T-cell lymphoma with aberrant expression of CD79a and CD20: A diagnostic pitfall. Mod Pathol 2001: 14: 105-10.
52. Theodorou I, Raphael M, Bigorgne C, et al. Recombination pattern of the TCR γ locus in human peripheral T-cell lymphomas. J Pathol 1994; 174: 233-42.

# มะเร็งต่อมน้ำเหลืองชนิด เพอริเฟอรอล ที-เซลล์ ที่มีความสัมพันธ์กับภาวะติดเชื้อไวรัสเอ็ปสไตน์-บาร์ ในระบบทางเดินอาหาร

*วิญญู มิตรานันท์, พบ*, วรรณรัตน์ แซ่ชั่น, วทม*, จินตนา ประดุจกาญจนา, วทม*, สุภาพร สุวิวัฒน์, วทม*, ซาโตมิ ทากาโอะ, วทม**, ทากาฟูมิ อิชิดะ, ปรด***

Peripheral T-cell lymphoma (PTCL) เป็นกลุ่มโรคที่พบมากในเอเชียและกลุ่มประเทศในอเมริกากลางและอเมริกาใต้ กลุ่มโรคนี้มีความสัมพันธ์กับการติดเชื้อไวรัสเอ็ปสไตน์-บาร์ (EBV) การศึกษานี้เพื่อประเมินผู้ป่วยที่มีโรค PTCL ในระบบทางเดินอาหาร โดยศึกษาอาการทางคลินิก การดำเนินโรค การเปลี่ยนแปลงทางพยาธิวิทยา ศึกษาในระดับโมเลกุลของภาวะติดเชื้อไวรัส EBV และศึกษาโคลน (clone) ของเซลล์มะเร็ง การศึกษานี้เริ่มตั้งแต่เดือนมกราคม พ.ศ. 2540 จนถึงเดือนธันวาคม พ.ศ. 2543 พบผู้ป่วยโรค PTCL ชนิดต่าง ๆ 129 ราย ในจำนวนนี้ 7 ราย (5.4%) พบรอยโรค PTCL ในระบบทางเดินอาหาร อาการสำคัญของผู้ป่วยที่มีรอยโรค PTCL ในระบบทางเดินอาหารคือไข้เรื้อรัง น้ำหนักลด ซีด ตับและม้ามโต ต่อมน้ำเหลืองโต มีรอยโรคในหลายอวัยวะและมีเลือดออกในทางเดินอาหาร การตรวจเลือดพบว่ามีการเพิ่มขึ้นของเอนไซม์ alkaline phosphatse และ lactate dehydrogenase และมีความผิดปกติในระบบการแข็งตัวของเลือด ผู้ป่วย 5 ใน 7 รายเสียชีวิตภายใน 4 เดือน หลังปรากฏอาการครั้งแรก ผู้ป่วยอีก 2 ราย หายขาดจากโรคหลังการได้รับยาเคมีบำบัด ผู้วิจัยได้แบ่งลักษณะของเซลล์มะเร็งเป็น 3 ประเภทคือ ประเภทที่มีเซลล์มะเร็งขนาดเล็ก ประเภทที่มีเซลล์มะเร็งขนาดปานกลางผสมกับขนาดใหญ่ และประเภทที่มีเซลล์มะเร็งขนาดใหญ่ ผลการตรวจโปรตีนที่อยู่บนผนังเซลล์ของเซลล์มะเร็งพบว่าเป็นชนิด LCA+, CD3+, CD15-, CD16-, CD30-, CD45RO+, CD57-, CD68-, EMA-, βF1-, granzyme B+, TIA+, และ p53+, สำหรับ CD4, CD8, CD56, CD20 บางรายให้ผลบวกและบางรายให้ผลลบ ตรวจพบ EBV RNA ในนิวเคลียสของเซลล์มะเร็งของผู้ป่วยทั้ง 7 ราย การตรวจโดยวิธี PCR ของ T-cell receptor (TCR) β และ/หรือ γ gene rearrangement ปรากฏว่าให้ผลบวกในผู้ป่วยทั้ง 7 ราย และการวิเคราะห์สาย DNA ซึ่งเป็นผลผลิตโดยวิธี PCR ของ TCR ดังกล่าวข้างต้น พบว่าลำดับเบสบนสาย DNA มีความคล้ายคลึงกับ human TCR germline gene มาก การวิจัยนี้แสดงว่าการเกิด PTCL ในระบบทางเดินอาหาร มีความสัมพันธ์กับภาวะติดเชื้อไวรัส EBV, เซลล์มะเร็งเป็น mature T-cell ที่แสดงโปรตีนของ NK-cell บางชนิดบนผนังเซลล์ กลุ่มเซลล์มะเร็งในผู้ป่วยแต่ละรายมาจากเซลล์เพียงหนึ่งโคลน และเป็นเซลล์ที่มีการ rearrange ของยีน TCR แล้ว

**คำสำคัญ** : ที-เซลล์ ลิมโฟมา, ไวรัสเอ็ปสไตน์-บาร์, ระบบทางเดินอาหาร, ยีนของ ที-เซลล์ รีเซฟเตอร์, โคลนของเซลล์มะเร็ง

**วิญญู มิตรานันท์, วรรณรัตน์ แซ่ชั่น, จินตนา ประดุจกาญจนา, สุภาพร สุวิวัฒน์, ซาโตมิ ทากาโอะ, ทากาฟูมิ อิชิดะ**
**จดหมายเหตุทางแพทย์ ฯ 2546; 86: 816-828**

* ภาควิชาพยาธิวิทยา, คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์, สงขลา 90110
** ภาควิชาชีววิทยา, มหาวิทยาลัยโตเกียว, โตเกียว, ญี่ปุ่น