Aggressive Peripheral T-Cell Lymphomas Containing Epstein-Barr Viral DNA: A Clinicopathologic and Molecular Analysis


The Epstein-Barr virus (EBV) has been shown to be associated with posttransplant lymphoma, Hodgkin’s disease, and T-cell lymphoma, in addition to African Burkitt’s lymphoma. In a retrospective study of 56 consecutive cases of T-cell lymphoma, EBV DNA was found by Southern blot and in situ DNA hybridization in 10 (20%) of 50 peripheral T-cell lymphomas, but in none of six cases of T-lymphoblastic lymphoma. Peripheral T-cell lymphomas containing EBV DNA could be subclassified into three categories according to histology and immunophenotypic studies: (1) T-cell lymphoma of the helper phenotype, five cases. Two cases had histologic features resembling angioimmunoblastic lymphadenopathy (AILD). (2) T-cell lymphoma of the cytotoxic/suppressor phenotype, four cases. AILD-like features could also be recognized in two cases. Reed-Sternberg-like giant cells were identified in three cases designated Hodgkin-like T-cell lymphoma. (3) Angiocentric T-cell lymphoma or lymphomatoid granulomatosis in one case, initially affecting the skin and nose; no T-cell subset could be defined. Six of the eight EBV DNA-positive patients tested for serum EBV antibodies had elevated titers of IgG antiviral capsid antigen (> 640) and/or early antigen (> 10). From combined studies of Southern blot hybridization by using EBV termini fragment probe and in situ DNA hybridization, the EBV genomes appeared to be clonotypically proliferated in the neoplastic T cells. The patients in all three groups usually had prolonged fever preceding the diagnosis, hepatosplenomegaly, an aggressive clinical course, and poor response to chemotherapy; nine died with a median survival of only 8 months. We propose that these EBV-associated aggressive T-cell lymphomas, like human T-cell leukemia/lymphoma virus-positive T-cell lymphoma, have characteristic clinicopathologic features and should be treated as a separate disease entity.

IN THE DISCOVERY OF THE Epstein-Barr virus (EBV) by Epstein et al in 1964,1 EBV has been found to be associated with African Burkitt’s lymphoma,2 posttransplant lymphoma,3 and undifferentiated carcinoma of the nasopharynx and thymus.4-6 A clonotypic proliferation of EBV has been demonstrated in the neoplastic cells of these malignancies, suggesting a causative role of EBV in the tumorigenesis.7-9 Recently, two additional human lymphoproliferative neoplasms, i.e., Hodgkin’s disease and T-cell lymphoma,7,9 have also been shown to contain EBV genome in the tumor cells. Therefore, the role of EBV in the tumorigenesis of human neoplasms becomes increasingly important.

Taiwan is an endemic area of EBV-associated nasopharyngeal carcinoma (NPC).4 The majority of general population in Taiwan have been infected with EBV at an early age before 2 years.10,11 We have previously reported a relatively high frequency of peripheral T-cell lymphoma in Taiwan,12,13 as compared with that in western countries. Although sporadic cases of these T-cell lymphomas have been shown to be associated with human T-cell leukemia/lymphoma virus (HTLV-I),12,13 the causative agent of the majority of these T-cell lymphomas still remains obscure. Therefore, we retrospectively investigated the presence of EBV DNA in 56 consecutive tissue samples of T-cell lymphoma by Southern blot hybridization. For the EBV DNA-positive cases, the EBV genomes were verified by in situ DNA hybridization, to be present in the tumor cells. Furthermore, an EBV termini fragment probe was used to determine the clonality of EBV genomes in the neoplastic cells by Southern blot hybridization. The clinicopathologic features of these patients with EBV-associated T-cell lymphomas are analyzed and discussed.

MATERIALS AND METHODS

Tissue samples. A total of 123 examples of non-Hodgkin’s lymphoma, collected from 1984 through 1989, were available for complete histopathologic, immunophenotypic, and molecular studies in the Department of Pathology, National Taiwan University Hospital. Six cases were T-lymphoblastic lymphoma and 50 cases were classified as peripheral T-cell lymphoma. Five T-cell lymphomas have been previously shown to be associated with HTLV-1.14 The tumor tissues from the 56 cases of T-cell lymphoma constituted the base of this study. Their clinical features and laboratory data were reviewed and analyzed.

Histopathology and immunophenotypic studies. Parts of the removed tumor samples were fixed in B5-formalin solution and processed for hematoxylin-eosin (H&E) stain. The remainder of the tissues were snap-frozen immediately in isopentane/liquid nitrogen and stored at −70°C until used.

For immunophenotypic studies, sections were cut at 6 μm and then stained with a panel of B- and T-cell-specific monoclonal antibodies (MoAbs) including CD19, CD20 (Coulter, Hialeah, FL), CD2, CD3, CD4, CD8 (Ortho, Raritan, NJ), and Tac (CD25), Ia (HLA-DR), transferrin receptors (Becton Dickinson, Mountain View, CA), and Ki-1 (CD30) (Dako, Santa Barbara, CA) by an avidin-biotin-complex peroxidase method.15 Determination of EBV receptors on lymphoid cells was performed using an MoAb against the C3d receptor (CD21) (Sera-
The diagnosis of T-cell lymphoma, based on the morphology and immunophenotypic studies, was made and classified as previously described. The clonality of these T-cell lymphomas was further investigated by Southern blot hybridization as described below.

Serologic tests of antibodies to EBV. Serologic tests of antibodies against IgG and IgM class EBV-viral capsid antigen (VCA), early antigen (EA), and EB nuclear antigens (EBNA) were performed using the method previously described on 8 of 10 EBV DNA-positive cases and also on 18 EBV DNA-negative lymphoma patients who had sera available for studies. A titer of IgG anti-VCA higher than 640, the positivity of IgM anti-VCA, or a titer of IgG anti-EA higher than 10 was regarded as significantly elevated based on the background data in the general populations in Taiwan. 

Table 1. Histopathologic and Immunophenotypic Studies of 10 Cases of EBV-Associated Peripheral T-Cell Lymphoma

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age/Sex (y)</th>
<th>Pathologic Diagnosis</th>
<th>Immunophenotypic Studies</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>CD2</td>
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<tr>
<td>Group A: Helper T-cell lymphoma</td>
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<tr>
<td>1</td>
<td>9/M</td>
<td>IBLt</td>
<td>+</td>
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<tr>
<td>2</td>
<td>40/M</td>
<td>Large cell</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>78/M</td>
<td>IBL</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>25/M</td>
<td>IBLt</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>5/M</td>
<td>Small/medium</td>
<td>+</td>
</tr>
<tr>
<td>Group B: Cytotoxic/suppressor T-cell lymphoma</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>31/F</td>
<td>IBL</td>
<td>+</td>
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<tr>
<td>7</td>
<td>7/M</td>
<td>IBLt (Hodgkin-like)</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>13/M</td>
<td>IBLt</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>62/M</td>
<td>IBL (Hodgkin-like)</td>
<td>+</td>
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<tr>
<td>Group C: Angioinvasive T-cell lymphoma</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>10</td>
<td>33/M</td>
<td>Angioinvasive (medium-sized)</td>
<td>+</td>
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</tbody>
</table>

Abbreviations: IBL, immunoblastic-type lymphoma.
*CD21 represents EBV the receptor, C3d.
1Cases with features of AILD.
Fig 2. EBV-associated CD4 phenotype T-immuno-blastic lymphoma (H-E, original magnification 400).

Fig 3. Hodgkin-like T-cell lymphoma of CD8 phenotype with the features of angioimmunoblastic lymphadenopathy. RS-like giant cells were noted against a background of atypied lymphocytes (H-E, original magnification 400).

Fig 4. Southern blot analysis of the rearrangement of the TCR-β gene using BglII-digested C_{β} fragment as the probe. Rearrangement of TCR-β gene could be demonstrated in cases 1 through 9, but case 10 (a case of angiocentric T-cell lymphoma) showed an absence of the TCR-β gene rearrangement using EcoRI, BamHI, and HindIII. For case 6, no rearrangement was observed in EcoRI-digested DNA, but the rearrangement of TCR-β could be clearly demonstrated using the BamHI restriction enzyme. Raji, a B-cell Burkitt's lymphoma line DNA, represents a germline control. Arrows indicate rearranged bands, and bars indicate germline bands.
DNA hybridization studies. The following strategy was used to perform DNA hybridization studies. Tumor DNA was first screened for the presence of EBV DNA by Southern blot hybridization. In EBV DNA-positive cases, rearrangements of the T-cell receptor-β (TCR-β) and Ig genes were further studied to investigate their clonality. In situ DNA hybridization was then followed to determine whether the EBV genomes were in the tumor cells or nonmalignant cells. Finally, to investigate the clonality of EBV genomes in the neoplastic cells, an EBV termini fragment probe was used for Southern blot hybridization.

Tumor-tissue DNA was extracted from frozen specimens of all 56 patients with T-cell lymphoma. For the demonstration of the presence of EBV DNA, 10 μg of tumor DNA was digested with BamHI restriction endonuclease (Boehringer, Mannheim, Germany). The digested DNA was then loaded onto a 0.8% agarose gel, electrophoresed, and blotted onto a Zeta-probe nylon membrane (AMF; Canno, Meriden, CT). After baking for 2 hours, the membrane was prehybridized and hybridized with the probes of EBV BamHI-A and -W fragments (kindly provided by Prof J.L. Strominger, Harvard University, Cambridge, MA) at a high radioactivity of 1 to 3 × 10^6 cpm/μg. For study of TCR-β and Ig gene rearrangement, tumor DNA was digested with restriction endonucleases BamHI, EcoRI, and HindIII, and hybridized with a probe for the TCR-β gene (BglII-digested Cβ3 fragment) and the JH fragment of the Ig gene (Oncor, Gaithersburg, MD).

For clonality analysis of EBV genomes in neoplastic cells, tissue or cell DNAs were digested with BamHI endonuclease, and an EBV-termini fragment probe (EcoRI-Dhet fragment, 13 kb; kindly provided by Dr Nancy Raab-Traub at University of North Carolina, Chapel Hill) was used for Southern blot hybridization. An EBV-containing Burkitt's lymphoma cell line Raji, two EBV-transformed lymphoblastoid cell lines (LBL-1 and LBL-2), and two EBV DNA-containing NPC tumor tissues were used as control specimens. The EcoRI-Dhet termini fragment contains a segment of EBV BamHI-A region and Bam Nhct that includes the unique region N as well as the terminal repeats. In clonotypic proliferation of EBV genomes or neoplastic conditions, there were usually two hybridization bands: one invariant band at around 12 kb and the other variant band usually shorter than 12 kb, corresponding to the EBV BamHI-A region and the clonotypically proliferated variable terminal region, respectively. In EBV-transformed lymphoblastoid cell lines or nonneoplastic conditions, multiple hybridization bands were usually detected, indicating a polyclonal proliferation or a replicative status of EBV genomes.

In situ DNA hybridization was performed on all 10 EBV DNA-positive and another 10 EBV DNA-negative tumors accord-
ing to the method of Brigati et al\textsuperscript{12} using the biotinylated EBV-W probe (Enzo). EBV DNA-containing Raji cell line and a tumor tissue of EBV-associated NPC were simultaneously included as positive controls. Negative controls included an EBV DNA-negative BJAB Burkitt’s lymphoma line (kindly provided by Prof E. Kieff, Harvard Medical School, Boston, MA) and an HTLV-I-positive EBV-negative MT-2 cell line. Briefly, the cut sections of freshly frozen tumor tissues or cytospinned smears of cell lines were fixed in methanol/glacial acetic acid for 10 minutes, air dried, and dipped in 3% H\textsubscript{2}O\textsubscript{2} for 30 minutes. The sections were then washed with 2X SSC (sodium chloride-sodium citrate), and dipped in avidin (1 mg/mL) and RNase (200 pg/mL) sequentially. After passing through a series of ascending ethanol, 10 to 20 µL of the biotinylated probe was applied to the sections in a humidified chamber, heated at 92 to 94°C for 1 to 1.5 minutes for denaturation. The slides were then transferred to a 37°C incubator for 1 hour. After incubation, the sections were immersed with 2X SSC, and 1 drop of posthybridization reagent was applied to each section for 10 minutes. The sections were then washed and an avidin-biotin-peroxidase complex was applied, and incubated in 37°C for 30 minutes. After incubation, the reaction was developed with filtered diaminobenzidine containing 0.03% H\textsubscript{2}O\textsubscript{2} for 3 to 25 minutes. The sections were then counterstained with hematoxylin, dehydrated, mounted with permount, and read under a light microscope. In EBV DNA-containing specimens, granular staining indicative of EBV DNA could be detected in the nuclei.

RESULTS

Survey of EBV DNA in T-cell lymphoma. Among the 56 cases of T-cell lymphoma, EBV DNA was detected in 10 (20%) of the 50 peripheral T-cell lymphomas, but in none of the six T-lymphoblastic lymphoma samples by Southern blot hybridization (Fig 1). There was a complete correlation of the results as studied by using EBV BamHI-A and -W fragments as probes, located at around 12 kb and 3 kb, respectively, as a single hybridization band. The intensity of the hybridization bands was comparable with that of Raji and B95.8 lines in eight samples, while samples had a relatively weak intensity; both weak signals occurred in tissue samples showing Hodgkin-like histology (cases 6 and 7). Five tumors, which had been previously shown to contain HTLV-1, were negative for EBV DNA. Therefore, a coexistence of EBV and HTLV-1 was not found.

Histopathology and immunophenotype studies. The 10
cases of EBV DNA-positive T-cell lymphomas were classified into the following three groups based on the histopathology and immunophenotypic studies (Table 1).

Five tumors were shown to be of the T-helper/inducer phenotype expressing pan-T markers and CD4 (group A), and four patients were of the T-cytotoxic/suppressor phenotype expressing pan-T markers and CD8 (group B). The histology of the five patients in group A was immunoblastic type (Fig 2) in three, large convoluted cell in one, and medium cell in one. Two cases had characteristic histologic features resembling angioimmunoblastic lymphadenopathy (AILD). Tumor necrosis was evident in two cases; one showed characteristic individual cell necrosis with scattered necrotic debris in the background. All four cases in group B were classified as immunoblastic lymphoma. AILD-like features could also be recognized in two cases. Three of the four tumors contained Reed-Sternberg (RS) or RS-like multinucleated giant cells (Fig 3) and could be confused with mixed cellularity type Hodgkin’s disease. However, they were classified as non-Hodgkin’s lymphoma because of atypical lymphoid cells in the background. One patient (group C) had a characteristic angiocentric nature of tumor cells initially involving skin of the penis, and later involving the nose. The tumor cells were of medium size in this case. This case would be classified as lymphomatoid granulomatosis. No T-cell subset can be defined, although pan-T cell markers such as CD2, CD3, and CD7 could be shown in most tumor cells.

The expression of EBV receptor CD21 was seen only in scattered cells, which were difficult to identify as neoplastic cells in all 10 cases. In control sections, the expression of CD21 could be clearly detected in the follicular center cells and scattered interfollicular cells.

Rearrangements of TCR and Ig genes. Rearrangement of the TCR-β chain gene was shown in all nine tumors in groups A and B (Fig 4), including the three cases of Hodgkin-like T-cell neoplasm. No rearrangement could be detected in EcoRI-digested DNA for case 6, but rearrangement of the TCR-β gene could be clearly demonstrated in the BamHI-digested blot. However, a DNA sample from the penile tumor of the patient in group C showed a germline pattern of TCR-β gene after analyses using the three restriction endonucleases EcoRI, Bam HI, and HindIII. All 10 cases showed a germline pattern of Ig gene by using the JH fragment as a probe (data not shown).

In situ DNA hybridization for EBV DNA. In situ DNA hybridization study using biotinylated EBV-W fragment probe showed positive EBV granules in tumor cell nuclei in positive control specimens from a Raji cell line and an NPC tumor tissue, and also in all 10 cases of EBV DNA-positive T-cell lymphoma (Fig 5). The percentage of EBV-positive cells in each tumor sample varied from case to case, ranging from 20% to around 100%, corresponding to the proportion of neoplastic T cells in each case. In the cases of Hodgkin-like T-cell lymphoma, granules indicative of EBV hybridization could be demonstrated in the nuclei of RS-like neoplastic giant cells (Fig 5D). EBV DNA-negative control specimens from BJAB and MT-2 cell lines and all 10 EBV DNA-negative T-cell lymphomas showed no detectable nuclear EBV DNA granules.

Clonotypic analysis of EBV genomes in the neoplastic cells. Southern blot hybridization analysis of BamHI-digested tissue or cell line DNA by using EBV termini EcoRI-Dhet fragment probe (Fig 6) showed that the lymphoblastoid cell lines (LBL-1 and LBL-2) had multiple hybridization bands, representing a polyclonal proliferation or linear forms of
EBV genomes in these EBV-transformed lymphoblastoid cells. Blots of BamHI digests of DNAs from NPC tumor tissues (NPC-1 and NPC-2) and most cases of EBV-associated T-cell lymphoma showed two hybridization bands: one invariant band at approximately 12 kb corresponding to the BamHI-A hybridization region (Fig 1) (the EcoRI-Dhet fragment probe includes a fragment of EBV BamHI-A region), and one variant shorter band that represents the differential clonal proliferation of EBV terminal region. The EBV-containing Raji cell line and three cases of EBV-associated T-cell lymphoma (cases 1, 3, and 10) showed only one hybridization band, suggesting that the clonal hybridization bands of the EBV terminal region have similar molecular size and are superimposed on the EBV BamHI-A region, or a deletion or mutation exists. Subgenomic fragment probe containing exclusively the terminal region may be indicated to clarify this point. These results suggested that a clonotypic proliferation of EBV genomes occurred in the neoplastic T cells in all 10 cases of EBV-associated T-cell lymphoma.

Pertinent clinical data. The age, sex, and clinical presentations of the 10 patients with EBV-associated T-cell lymphoma are listed in Table 2. Characteristic findings were the frequent constitutional symptoms such as prolonged fever of unknown origin preceding the diagnosis, night sweats, and loss of body weight (nine patients); neck or systemic lymphadenopathy (10 patients); and hepatosplenomegaly (six patients). Other manifestations included

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age/Sex</th>
<th>Pathologic Classification</th>
<th>Clinical Manifestations</th>
<th>Pertinent Laboratory Data</th>
<th>Clinical Staging</th>
<th>Treatment</th>
<th>Survival (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A: Helper T-cell lymphoma</td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>9/M</td>
<td>IBL</td>
<td>Fever 3 mo, lymphadenopathy, hepatomegaly</td>
<td>Anemia, cytopenia, LDH ↑ (850), hypergamma globulinemia</td>
<td>III</td>
<td>CHOP</td>
<td>6(D)</td>
</tr>
<tr>
<td>2</td>
<td>40/M</td>
<td>Large cell</td>
<td>Fever 2 mo, night sweating, skin nodules, lymphadenopathy</td>
<td>Anemia, LDH ↑ (2,040), abnormal LFT, hypergamma globulinemia</td>
<td>III</td>
<td>CHOP + retinoids, bone marrow transplantation</td>
<td>8(D)</td>
</tr>
<tr>
<td>3</td>
<td>78/M</td>
<td>IBL</td>
<td>Loss of body weight, lymphadenopathy, diffuse lung lesions</td>
<td>Hypergamma globulinemia</td>
<td>IV</td>
<td>COP</td>
<td>12(D)</td>
</tr>
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<td>4</td>
<td>25/M</td>
<td>IBL</td>
<td>Fever 3 wk, jaundice, lymphadenopathy, hepatosplenomegaly</td>
<td>Abnormal LFT, bone marrow (+)</td>
<td>IV</td>
<td>CHOP</td>
<td>5(D)</td>
</tr>
<tr>
<td>5</td>
<td>5/M</td>
<td>Small/medium</td>
<td>Fever 3 mo, lymphadenopathy, hepatosplenomegaly</td>
<td>Anemia, thrombocytopenia, abnormal LFT, hypergamma globulinemia</td>
<td>IV</td>
<td>CHOP + bleomycin</td>
<td>3(D)</td>
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<tr>
<td>Group B: Cytotoxic/suppressor T-cell lymphoma</td>
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<td></td>
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<tr>
<td>6</td>
<td>31/F</td>
<td>IBL (Hodgkin-like)</td>
<td>Fever 9 mo, headache, blurred vision, lymphadenopathy, hepatomegaly, multiple brain lesions</td>
<td>Pancytopenia, abnormal LFT, LDH ↑ (1,681) bone marrow (+)</td>
<td>IV</td>
<td>COP</td>
<td>19(D)</td>
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<td>7</td>
<td>6/M</td>
<td>IBL (Hodgkin-like)</td>
<td>Fever 6 mo, lymphadenopathy, hepatosplenomegaly</td>
<td>Pancytopenia, abnormal LFT, LDH ↑ (1,884) bone marrow (+)</td>
<td>IV</td>
<td>CHOP + bleomycin</td>
<td>13(D)</td>
</tr>
<tr>
<td>8</td>
<td>13/M</td>
<td>IBL</td>
<td>Fever 1 mo, jaundice, lymphadenopathy, hepatosplenomegaly</td>
<td>Anemia, LDH ↑ (12,800), abnormal LFT, hypogamma globulinemia</td>
<td>IV</td>
<td>COP</td>
<td>3(D)</td>
</tr>
<tr>
<td>9</td>
<td>62/M</td>
<td>IBL (Hodgkin-like)</td>
<td>Cervical lymphadenopathy, lung nodules</td>
<td>All within normal limits</td>
<td>IV</td>
<td>CHOP</td>
<td>9(A)</td>
</tr>
<tr>
<td>Group C: Angioinvasive T-cell lymphoma</td>
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<tr>
<td>10</td>
<td>33/M</td>
<td>Angioinvasive, medium</td>
<td>Fever 1 y, penile ulceration, nasal lesions</td>
<td>Anemia, LDH ↑ (548), abnormal LFT</td>
<td>III</td>
<td>Leukeran, prednisone</td>
<td>14(D)</td>
</tr>
</tbody>
</table>

Abbreviations: IBL, immunoblastic lymphoma; CHOP, cyclophosphamide + Adriamycin + Oncorvin + prednisolone; A, alive; D, died.
skin lesions (two patients), lung lesions (two patients), and relapsing ulceration of penile skin and midline nasal lesion in the patient in group C.

Laboratory examinations showed anemia in six patients at presentation, and abnormal liver functions were found in seven patients. Six patients had elevated lactic dehydrogenase (LDH). Two patients had profound hypogammaglobulinemia; both belonged to group B cytotoxic-suppressor T-cell lymphoma, and three had polyclonal hypergammaglobulinemia; all belonged to group A patients. Bone marrow involvement by the tumor was noted in two patients at presentation and during the clinical course in five patients. All patients had advanced stage III/IV diseases.

Serologic tests of antibodies to EBV. Among the eight EBV DNA-positive lymphoma patients who received serologic tests of antibodies to EBV, six had elevated titers to EBV-VCA IgG (>640) and four to EBV-EA IgG (10 to 40) (Table 3), which were interpreted as an active EBV infection based on the background data in the general populations in Taiwan. None of them had elevated IgM class anti-VCA. All eight patients were EBNA-positive, indicating a past or remote infection. One patient with CD8 phenotype lymphoma (patient 8) had fluctuated titers of antibodies; the titers of anti-EBV-VCA IgG and anti-EA IgG were low at initial presentation when profound hypogammaglobulinemia (IgG 320 mg%, IgM 50 mg%, IgA 30 mg%) was also noted. After chemotherapy, the titers of serum anti-EBV-VCA IgG elevated from 1:160 to 1:1,280, and anti-EBV-EA IgG from 1:10 to 1:40. By the same time, the serum Ig recovered to IgG 750 mg%, IgA 100 mg%, and IgM 80 mg%. Therefore, the CD8+ tumor cells in this case appeared to have a suppressive effect on the production of Ig, and the immune response to EBV infection may also have been inadequate or suppressed in this case. Among the 18 EBV DNA-negative T-cell lymphoma patients studied, none of them had a titer of IgG anti-VCA higher than 640 or a titer of anti-EA higher than 10, although anti-EBNA was positive in all patients.

Clinical progression and survival. All 10 patients had aggressive clinical courses; nine died with a median survival of 8 months (range, 3 to 19 months). The response to chemotherapy was incomplete or transient with only brief remission intervals. As compared with the median survival of 18 months in the group of EBV-negative and HTLV-1-negative T-cell lymphoma, the prognosis is significantly worse (P < .05) and closely approached that of HTLV-1-positive T-cell lymphoma (7 months in this series).

**DISCUSSION**

The EBV was not previously shown to be associated with T-cell lymphoma until Jones et al first demonstrated the presence of EBV genomes in the tumor cells of CD4+ peripheral T-cell lymphoma in three patients with chronic EBV infection. We later confirmed the observation and found that EBV can also contribute to the development of CD8+ (cytotoxic/suppressor) peripheral T-cell lymphoma. This communication represents a systematic survey on the association of EBV with T-cell lymphoma by using Southern blot and in situ DNA hybridization studies. We showed that EBV was closely associated with a spectrum of peripheral T-cell lymphomas, in addition to Burkitt's lymphoma, posttransplant B-cell lymphoma, and Hodgkin's disease.

The possibility that these T-cell proliferations in the current series represent a reactive process to an EBV infection, or infectious mononucleosis, can be reasonably excluded by the demonstration of clonal rearrangement of the TCR-β chain genes in tumor tissues from all nine patients in groups A and B. We did not detect the rearrangement of the TCR-β or Ig gene in the patient with angiocentric T-cell lymphoma or lymphomatoid granulomatosis in group C. The same observation of an absence of the TCR-β gene rearrangement in a particular group of peripheral T-cell lymphomas affecting the nose and nasopharyngeal regions has been previously reported, and a hitherto undefined lineage of T-cell subsets or probably a T-γ/δ
hybridization provides strong evidence that EBV can infect neoplastic T cells. The clonality analysis of EBV genomes by Southern blot hybridization using the EBV termini fragment probe in this series further shows a clonotypic proliferation of EBV genomes in the neoplastic T cells, as was previously demonstrated in NPC and B-cell lymphoma. Although a previous history of chronic EBV infection was not documented in our patients, the prolonged fever preceding the diagnosis of lymphoma, the elevated titers of IgG class anti-EBV VCA, and the positivity of EBNA in most of our cases tested for EBV antibodies support such an assumption that a chronic EBV infection did occur in some of our patients, as was previously observed in the three cases of EBV-associated T-cell lymphoma. However, in this study we did not clearly show the expression of EBV receptor, C3dR (CD21), on the neoplastic cells. In a recent report of EBV-associated nasal T-cell lymphoma, the neoplastic T cells also did not express detectable CD21 on the cell membrane. Therefore, the mechanism of the infection of these neoplastic T cells by EBV remains unknown. EBV receptor has been shown to be expressed in a low percentage of neoplastic cells in CD4+ T-cell lymphoma. There exists a possibility that the expression of CD21 on these neoplastic T cells may be transient, cell-cycle dependent, or at a low level, such as that demonstrated in epithelial cells or nasopharyngeal carcinoma.

The histology of EBV-associated peripheral T-cell lymphoma is heterogeneous, in contrast to the homogeneous morphology of EBV-associated Burkitt's lymphoma. The heterogeneity of histology possibly results from the effects of lymphokines released by the transformed or activated T cells. Two characteristic histologic features are recognized in this series. One is the presence of AILD-like patterns in four cases, two each in groups A and B, the so-called AILD-like T-cell lymphoma. The second feature is the presence of RS and RS-like giant cells in three cases of group B patients. RS or RS-like giant cells have been shown in various types of EBV-associated lymphoproliferative disorders, such as infectious mononucleosis, Hodgkin's disease, and Hodgkin-like T-cell lymphoma.

The association of EBV with lymphomatoid granulomatosis affecting the skin and nose in the patient in group C is interesting considering the relative high frequency of angiocentric T-cell lymphoma in Hong Kong and Taiwan (Hsieh HC and Su II, unpublished observation, 1990), both regions that have been well-known endemic areas of EBV-associated nasopharyngeal carcinoma. Lymphomatoid granulomatosis has been previously observed to be associated with EBV. A recent observation has further demonstrated a causal association of EBV with nasal T-cell lymphoma or lethal midline granuloma. Further large-scale study is mandatory to clarify the unique role of EBV in this group of nasal T-cell lymphoma or lymphomatoid granulomatosis.

The clinical behavior of EBV-associated T-cell lymphoma in all patients was aggressive with a median survival of only 8 months, although intensive chemotherapy had been administered for most patients. The prognosis is significantly worse than that of EBV DNA-negative T-cell lymphoma in this series (18 months), and closely approaches that of HTLV-1–positive T-cell lymphoma (7 months). There are frequent systemic symptoms and signs such as fever and hepatosplenomegaly in most patients. A characteristic finding was the prolonged fever of unknown origin preceding the lymphoma diagnosis. These clinical manifestations are similar to those of the three EBV-associated T-cell lymphoma cases reported previously and some of the high-grade T-immunoblastic lymphomas.

In summary, we have demonstrated and characterized a pathologic spectrum of EBV-associated T-cell neoplasms, the patients of which usually have prolonged fever, frequent constitutional symptoms/signs, an aggressive clinical course, and a poor prognosis. The EBV has been shown to be clonotypically proliferated in the neoplastic T cells and may play a causal role in the lymphomagenesis. Therefore, we propose that these EBV-associated T-cell lymphomas, like the HTLV-1–positive adult T-cell lymphoma/leukemia, should be treated as a separate disease entity. The association of EBV infection with T-cell lymphoma, in addition to HTLV-1, may help to explain the high frequency of T-cell malignancies in Taiwan.

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