A novel cell line, FL-18, was established from the pleural effusion of a patient with follicular small cleaved cell lymphoma. At the same time, an Epstein-Barr virus (EBV) nuclear antigen (EBNA)-positive cell line, FL-18-EB, was established from the EBV-infected culture of the same pleural effusion cells. Both cell lines had the same monoclonal surface immunoglobulin (IgGε), and they had the same karyotype as that of the fresh pleural effusion cells in which a reciprocal translocation between the long arm of chromosomes 14 and 18 ([t(14;18)(q32;q21)] was detected. Gene rearrangement analysis of immunoglobulin heavy-chain gene (JH) and κ light-chain gene (Jκ) showed the same rearranged configurations in the two cell lines; however, some morphological and phenotypic differences were found. The FL-18-EB cells, which were morphologically similar to common EBNA-positive lymphoblastoid cell lines of normal B cell origin at the initial phase of culture, were larger than the FL-18 cells and contained multinucleated giant cells. The FL-18 cells lacked cytoplasmic immunoglobulin and were positive for common acute lymphoblastic leukemia antigen (CALLA), whereas the FL-18-EB cells had cytoplasmic immunoglobulin and were negative for CALLA. Thus, the phenotype of FL-18-EB seems to be a result of a shift by EBV infection to a more mature stage in the B cell differentiation pathway than that of FL-18. The paired availability of EBV-free and EBV-infected cell lines of a neoplastic clone is unique and valuable in considering EBV infectibility of neoplastic B cells and resultant phenotypic changes.

Preparation of EBV, infection in vitro, and establishment of the cell lines. Preparation of EBV and infection in vitro were performed as previously reported. Briefly, the culture supernatants of B95-8 cells were centrifuged for two hours at 15,000 g. The pellet was diluted with a nonconcentrated culture supernatant. The pleural effusion cells were washed twice in phosphate-buffered saline and then were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS). Two weeks later, continuous cell growth was observed, and the established cell line was designated FL-18. At the same time, 10⁶ pleural effusion cells were incubated with 0.4 mL of the EBV for 90 minutes at 37°C. The infected cells were washed twice and cultured in the same manner as the untreated culture. They also showed continuous growth and were found to be positive for EBNA 2 months later. This EBNA-positive cell line was designated FL-18-EB. The FL-18 cells were infected in vitro in the same way as the fresh pleural effusion cells. The presence or absence of EBNA-positive cells was tested once a week until 3 months after the infection. The same experiment was repeated three times.

Cytotoxic examination, cell surface marker analysis, and EBNA. The pleural, FL-18, and FL-18-EB cells were examined with peroxidase (POX), α-naphthyl acetate esterase (NAE), naphthol AS-D chloroacetate esterase (CAE), and PAS. Spontaneous rosette formation with sheep erythrocytes (E rosette), receptors for the Fe portion of IgG (Fcγ receptor) and receptors for complement (C3b and C3d receptor) were examined as previously described. Surface and cytoplasmic immunoglobulin (Ig) was tested by the

MATERIALS AND METHODS

Case report. A 68-year-old Japanese male noted cervical lymph node swelling in August 1980. Lymph node biopsy in February 1982 revealed follicular small cleaved cell lymphoma. He received combined chemotherapy and achieved complete remission. A pleural effusion developed in July 1984. Cell lines were established from the culture of the pleural effusion cells collected at this time. The patient gave informed consent. He died in November 1984.

Preparation of EBV, infection in vitro, and establishment of the cell lines. Preparation of EBV and infection in vitro were performed as previously reported. Briefly, the culture supernatants of
direct immunoflourescence method. Reactivities with monoclonal antibodies were tested by the indirect immunofluorescence method. Monoclonal antibodies used in this study consisted of OKIa-1 for the HLA-DR antigen, B1,11 B2,18 J5 for common acute lymphoblastic leukemia antigen (CALLA),19 Leu-1,20 PCA-1,21 EBVCS-4,22 EBVCS-5,22 and OKT9.23 EBNA was tested according to the method of Reedman and Klein.24

Cytogenetic study. The pleural effusion cells were processed after short-term culture (24 hours) in RPMI 1640 medium supplemented with 20% FCS at 37°C. The cells were harvested after a 60-minute incubation with Colcemid and then treated with 0.075 mol/L KCl for 20 minutes before they were fixed in methanol acetic acid (3:1). Metaphases were banded by trypsin-Giemsa (G-band).25 Chromosome analysis of the cultured FL-18 and FL-18-EB cells was done 6 months after the initiation of the culture. Metaphase preparation was performed when the cells showed exponential proliferation. Processing and banding methods were the same as those of the pleural effusion cells.

Gene rearrangement analysis of Ig heavy chain and light chain. Genomic DNA was extracted from the THP-1 (for control), FL-18, and FL-18-EB cells as previously described26 and digested with BamHI. The digested DNA was fractionated by agarose gel electrophoresis and transferred to nitrocellulose paper according to the method of Southern.27 The 3.4-kilobase (kb) EcoRI-Hind III fragment of the joining region of the heavy-chain gene and the 2-kb ScaI-ScaI fragment of the joining region of the γ chain gene were used as Jγ and Jx probes. The nick-translated 32P-labeled probes were hybridized to the nitrocellulose filters and visualized on autoradiograms.

RESULTS

Morphological findings. Cytocentrifuged smears of the FL-18 and FL-18-EB cells are shown in Fig 1. The pleural effusion cells were small cleaved lymphoid cells that were morphologically the same as the lymph node cells with a diagnosis of follicular lymphoma. The FL-18 cells were also small to medium in size. The FL-18-EB cells, however, were larger than the FL-18 cells and contained multinucleated giant cells. The FL-18-EB cells were indistinguishable from the cells of common lymphoblastoid cell line of normal B cell origin at the early phase of the culture because the FL-18-EB cells formed firm clumps and exhibited pseudopods.28

Cytochemical examination, surface marker analysis, and EBNA. Table 1 shows the results of cytochemistry and cell surface phenotype including reactivities with the monoclonal antibodies and EBNA. The pleural, FL-18, and FL-18-EB cells were all negative for POX, NAE, or CAE. The FL-18-EB cells were positive for PAS, although the pleural effusion cells were negative. The fresh pleural effusion cells were positive for HLA-DR and B1 and were negative for E rosette or Leu-1, whereas surface Ig was not detected. B2 was not tested in the pleural effusion cells.

![Image A](image1.png)  
![Image B](image2.png)

**Fig 1.** May-Giemsa stain of cytocentrifuged smears of (A) FL-18 and (B) FL-18-EB.

<table>
<thead>
<tr>
<th>Markers</th>
<th>Pleural Effusion Cells (%)</th>
<th>FL-18 (%)</th>
<th>FL-18-EB (%)</th>
</tr>
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<tbody>
<tr>
<td>POX</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NAE</td>
<td>-</td>
<td>-</td>
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<td>CAE</td>
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</tr>
<tr>
<td>PAS</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>E rosette</td>
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<td>0</td>
</tr>
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<td>Fcy receptor</td>
<td>17</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>C3b receptor</td>
<td>&lt;1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>C3d receptor</td>
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<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Surface Ig</td>
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</tr>
<tr>
<td>κ</td>
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<td>15</td>
</tr>
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<td>σ</td>
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<td>Cytoplasmic Ig</td>
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<tr>
<td>HLA-DR</td>
<td>80</td>
<td>99</td>
<td>70</td>
</tr>
<tr>
<td>B1</td>
<td>87</td>
<td>99</td>
<td>77</td>
</tr>
<tr>
<td>B2</td>
<td>NT</td>
<td>8</td>
<td>49</td>
</tr>
<tr>
<td>CALLA</td>
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<td>67</td>
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</tr>
<tr>
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<tr>
<td>PCA-1</td>
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</tr>
<tr>
<td>EBNA</td>
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</tr>
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</table>

Abbreviation: NT, not tested.

Table 1. Cytochemical and Immunologic Markers of Pleural Effusion Cells, FL-18, and FL-18-EB
18-EB cells. The fresh lymphoma cells were hyperdiploid, and the modal chromosome number was 49 with gains of chromosomes 7, 12, and an X. They showed a reciprocal translocation between the long arms of chromosomes 14 and 18 [(t(14;18)(q32;q21)]. In addition, complex chromosome rearrangements involving nos. 8, 13, and 22 were observed. FL-18 also had a 14q+ marker chromosome that was the result of a translocation between nos. 14 and 18. The modal karyotype was the same as that of the pleural effusion cells. In some cells, however, additional abnormal chromosomes such as 6p−, 17q+, and Xp− were found. The modal karyotype of FL-18-EB was the same as that of the fresh lymphoma cells. The additional abnormal chromosomes observed in FL-18 were not found in FL-18-EB. Fifty percent of the karyotypes of FL-18-EB showed a tetraploid genome. None of the FL-18-EB cells showed a normal karyotype.

**DISCUSSION**

FL-18 is thought to be derived from the lymphoma cells in the pleural effusion of a patient with follicular small cleaved cell lymphoma because it carried the same karyotype as that of the pleural effusion cells and was negative for EBNA. Cytogenetically, FL-18 had a reciprocal translocation between the long arms of chromosomes 14 and 18 [(t(14;18)(q32;q21)], which is known to be specific for follicular lymphoma.29 The details of the karyotype of FL-18 have been reported previously.30 FL-18 had monoclonal surface Ig globulin (IgGκ), although no surface or cytoplasmic Ig was detected in the pleural effusion cells.

We also established an EBV-carrying cell line, FL-18-EB. It is unlikely that FL-18-EB is a common lymphoblastoid cell line derived from normal B cell in the pleural effusion because FL-18-EB had the same karyotype and monoclonal Ig as FL-18. Moreover, Ig gene rearrangement analysis
using $J_H$ and $J_k$ as probes revealed the same rearranged bands in the two cell lines, thereby indicating their clonal identity.

Several morphological and phenotypic differences were found between FL-18 and FL-18-EB. Unlike the FL-18 cells, the FL-18-EB cells were larger and contained multinucleated giant cells. At the early stage of passage, the FL-18-EB cells were morphologically indistinguishable from common lymphoblastoid cell lines of normal B cell origin. Some investigators have described similar changes accompanying EBV transformation of neoplastic B cells. Karande et al reported that EBV-transformed chronic lymphocytic leukemia cells were morphologically similar to common lymphoblastoid cell lines. Fresen and zur Hausen also reported that their EBV-infected BJAB cells contained multinucleated giant cells. In addition, the expression of the EBVCS-4 or -5-determined antigen can be regarded as a feature shared by common lymphoblastoid cell lines with FL-18-EB but not with FL-18. It should be noted that the antigen is not related specifically to EBV infection of the cell lines because Raji cells do not express this antigen.

The FL-18-EB cells expressed the B2 antigen, whereas the FL-18 cells hardly did. The C3d receptor detected with B2 is thought to be a receptor molecule for EBV. The augmentation of the C3d receptor on the FL-18-EB cells may be a result of EBV infection as has been described. Alternatively, EBV may have infected the C3d receptor-positive subpopulation of the fresh lymphoma cells, thereby leading to the generation of FL-18-EB, although the extent to which the fresh cells expressed the B2 antigen is unknown. The FL-18 cells lacked cytoplasmic Ig and were positive for CALLA, whereas the FL-18-EB cells had cytoplasmic Ig and did not express CALLA. It is generally thought that, in the differentiation from B cells to plasma cells, cytoplasmic Ig appears and surface Ig fades away. CALLA, which is expressed on most of the precursors of B cells such as common ALL and a part of B cells, disappears in the differentiation of B cells. Therefore, FL-18-EB seems to be closer to plasma cells than FL-18 is, although the FL-18-EB cells did not react with PCA-1, which is thought to be specific for plasma cells. These phenotypic differences are probably due to EBV infection itself rather than to the clonal difference of the two cell lines. To confirm the effect of EBV infection on the lymphoma cells, we tried to infect the FL-18 cells with EBV in vitro. No EBNA-positive cells were induced in any experiments. However, further studies are necessary to prove the absence of the infectibility.

The FL-18 and FL-18-EB cell lines may be of value for examining two isogenic cell lines at slightly different stages of maturation.

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Lymphoma cell line (FL-18) and Epstein-Barr virus-carrying cell line (FL-18-EB) obtained from a patient with follicular lymphoma: monoclonal derivation and different properties

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