Clinicopathologic, Immunophenotypic, and Immunogenotypic Analyses of Immunoblastic Lymphadenopathy-Like T-Cell Lymphoma

By Kensei Tobinai, Keisuke Minato, Tomoko Ohtsu, Kiyoshi Mukai, Yoshitoyo Kagami, Masanao Miwa, Shaw Watanabe, and Masanori Shimoyama

Immunoblastic lymphadenopathy (IBL)-like T-cell lymphoma is a distinct peripheral T-cell lymphoma, which closely resembles angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) and/ or IBL, but is characterized by focal or sheet-like proliferation of immunoblasts and pale cells of T-cell nature. In this report, 36 patients with IBL-like T-cell lymphoma were analyzed. The disease is clinically characterized by generalized lymph node swelling, hepatosplenomegaly, fever, skin rash, polyclonal hypergammaglobulinemia, marked male predominance, predilection for the elderly, and poor prognosis. There was no association with human T-cell leukemia virus type I or human immunodeficiency virus. IBL-like T-cell lymphoma may be divided into two categories (CD4+ type and CD8+ type) by surface marker analysis. It can also be divided into three categories on the basis of the histologic findings of distribution of morphologically recognizable tumor cells: nine cases of "inconspicuous type," six cases of "patchy type," and 21 cases of "diffuse type." Two cases of "inconspicuous type" converted later to "diffuse type." DNA hybridization analyses in the ten recent cases revealed that three of four "inconspicuous types" and five of six "diffuse types" showed clonal rearrangement of T-cell receptor beta chain gene without rearrangement of immunoglobulin heavy chain gene, providing strong evidence for clonal proliferation of T cells.

A group of systemic lymphoproliferative disorders have been described under various names such as immunodysplastic disease,1 angioimmunoblastic lymphadenopathy with dysproteinemia (AILD),2,3 immunoblastic lymphadenopathy (IBL),4 and lymphogranulomatosis X.5 These diseases were characterized clinically by generalized lymphadenopathy, hepatosplenomegaly, fever, skin rash, polyclonal hypergammaglobulinemia, and Coombs-positive hemolytic anemia. Histologically, involved lymph nodes were reported to show an obliteration of the normal lymph node architecture by a polymorphic cellular infiltrate including small lymphocytes, immunoblasts, and plasma cells together with a proliferation of arborizing small blood vessels. Despite a fatal outcome in most cases, AILD and IBL were reported as nonneoplastic lymphoproliferative disorders and considered to be abnormal hyperimmune B-cell disorders.

In 1979, we found focal neoplastic proliferation of atypical lymphoid cells consisting of immunoblasts, plasmacytoid cells, and so-called "pale cells" in the enlarged lymph nodes of such patients, whose clinical manifestations and histologic features were indistinguishable from those of AILD or IBL.

Immunophenotypic studies disclosed that most atypical lymphoid cells, namely immunoblasts, plasmacytoid cells, and pale cells, had T-cell markers. In addition, these patients showed fatal outcomes, as is common in malignant lymphoma. Therefore, we proposed a new disease entity, "IBL-like T-cell lymphoma," as a variant of peripheral T-cell lymphoma.6 The neoplastic cells from the six initial patients with IBL-like T-cell lymphoma were examined using monoclonal antibodies and were found to have CD8+ phenotype,8,9 however, some cases with CD4+ phenotype were reported from other institutions in Japan.9,10 Recently, molecular probes for immunoglobulin gene and T-cell antigen receptor gene have been used to aid in the cell lineage determination and identification of clonal populations in lymphoid malignancies.11,12 In the present study, clinical, histologic, and immunophenotypic analyses on 36 patients and immunogenotypic analysis on the ten recent patients with IBL-like T-cell lymphoma were performed to elucidate the nature of the disease.

MATERIALS AND METHODS

Diagnostic criteria and patients. As previously described,3,4,5,13 the diagnostic criteria of IBL-like T-cell lymphoma are as follows: histologically, normal lymph node architecture is obliterated, there are usually no germinal centers, and prominent proliferation of small blood vessels together with mixed cellular infiltration of small lymphocytes, plasma cells, histiocytes, and eosinophils are found. In addition, the presence of focal or sheet-like proliferations of atypical lymphoid cells such as immunoblasts, plasmacytoid cells, and in particular, pale cells should be found. These atypical lymphoid cells should have T-cell markers. Clinically, generalized lymph node swelling, hepatosplenomegaly, high fever, skin rash, and polyclonal hypergammaglobulinemia are present in most patients. According to the diagnostic criteria, 36 patients were diagnosed as having IBL-like T-cell lymphoma during the period from January 1978 to June 1987.

Morphology. Sections stained with hematoxylin, cosine, and periodic acid Schiff (PAS) were examined. Touch smears of biopsied lymph nodes or smears of aspirated lymph nodes stained with May-Giemsa were also examined.

Immunophenotypic analysis for suspended cells. Single cell suspensions of biopsied lymph nodes were used for the analysis. Spontaneous rosette-forming capacity (RFC) with sheep erythrocytes (E) was examined for all 36 cases. Surface immunoglobulin (S-Ig) and cytoplasmic immunoglobulin (C-Ig) were detected by

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direct immunofluorescence assay. For the 23 recent cases, indirect immunofluorescence assay using various monoclonal antibodies was performed by fluorescence microscopy and/or flow cytometry (Spectrum-III; Ortho Diagnostic Systems, Tokyo). Immune rosette assay using monoclonal antibodies and ox RBCs sensitized with anti-mouse IgG antibody (Immuno Biological Laboratory, Takasaki, Japan) was also performed in order to confirm the nature of rosette-forming cells in some cases. Monoclonal antibodies used in this study were CD2(9.6), CD3(OKT3), CD4(OKT4), CD5(10.2), CD8(OKT8), CD20(B1), and OKI1 for HLA-DR antigen.

Immunohistochemistry. The frozen tissues of the lymph nodes were stained with peroxidase-antiperoxidase or avidin-biotin complex methods using various monoclonal antibodies after paraformaldehyde-lysine-periodate fixation, as previously described. This analysis was performed in the eight most recent cases.

Immunogenotypic analysis. DNA was extracted from frozen lymph node cells and purified as previously described. DNA (10 μg) was digested with restriction enzymes, separated by electrophoresis in 0.8% agarose gel, and then transferred to activated nylon membranes according to the method of Southern. The DNA fragments on the membranes were hybridized with nick-translated 32P-labeled probes, and examined by autoradiography. The probes used in this study were JH probe and CT beta probe. The restriction enzymes used were HindIII for JH probe, and BamHI and EcoRI for CT beta probe. The immunogenotypic analyses were performed in the ten most recent cases.

Serological assay for anti-human T-cell leukemia virus type I (HTLV-I) antibody and anti-human immunodeficiency virus (HIV) antibody. Anti-HTLV-I antibody was examined by means of the particle agglutination test (PA test; Serodia-ATLA, Fujirebio Inc, Tokyo) and indirect immunofluorescence assay. Anti-HIV antibody was examined by enzyme-linked immunosorbent assay (ELISA; Organon Teknika, Holland) and Western blot assay as previously described.

Statistical analysis. Differences in the clinical characteristics and histologic findings between CD4-positive and CD8-positive groups were tested with Fisher's exact test. Survival curves were calculated by the method of Kaplan and Meier. Differences in the survival curves were tested by the generalized Wilcoxon test.

RESULTS

Clinical manifestations and laboratory findings. Clinical characteristics of 36 patients with IBL-like T-cell lymphoma are shown in Table 1 and Fig 1. Patients with IBL-like T-cell lymphoma tended to be elderly, ranging in age from 36 to 80 years old. Males predominated with a male-to-female ratio of 6:1. Only burned-out or fibrotic germinal centers were observed in five of the 27 patients examined had anti-HTLV-I antibody, and none of the 14 patients examined had anti-HIV antibody.

Histologic findings. Table 2 summarizes the histologic findings of the 36 patients with IBL-like T-cell lymphoma. Common structural changes were diffuse obliteration of normal lymph node architecture with prominent vascular proliferation, and almost complete disappearance of germinal centers. In seven of the 36 patients, germinal centers were found in part, but they were mostly burned-out or fibrotic. Proliferation of immunoblasts and plasma cells was found in about three-fourths of the cases. Infiltration of histiocytes and eosinophils was frequently seen, but the degree varied among patients.

The most characteristic finding was the proliferation of abnormal lymphoid cells with pale cytoplasm, so-called "pale cells," which were considered to be neoplastic cells. The nuclei of pale cells were usually medium-sized with nuclear irregularity and variable atypism. Occasional mitotic figures were noted in these pale cells. However, the degree of infiltration of pale cells was quite variable. Pale cells were conspicuous in 20 cases (55.6%). In seven of the remaining 16 cases, abnormal immunoblast-like cells with...
Fig 2. Histology of IBL-like T-cell lymphoma. (A) "Inconspicuous type": obliteration of normal lymph node architecture and proliferation of small blood vessels; small lymphoid cells and histiocytes dominated, but pale cells were not conspicuous. Three months later, the histology converted to that of "diffuse type" (B) (H&E, original magnification x 350). (B) "Diffuse type": together with prominent vascular proliferation, diffuse proliferation of pale cells is shown. Immunohistochemical analysis revealed the pale cells to show CD4 + phenotype (H&E, original magnification x 350). (C) "Patchy type": patchy distribution of pale cells together with vascular proliferation and a mixed cellular infiltrate (H&E, original magnification x 350). (D) "Diffuse type": diffuse proliferation of pale cells. Immunophenotypic analysis disclosed that the tumor cells of this case showed CD8 + phenotype (H&E, original magnification x 700).

basophilic cytoplasm were prominent, and they were regarded as lymphoma cells.

Based on the distribution of morphologically recognizable tumor cells, such as pale cells and immunoblast-like cells, the disorder was divided into three categories: nine cases of "inconspicuous type" (Fig 2A), six of "patchy type" (Fig 2C), and 21 of "diffuse type" (Fig 2B and D). Two cases of "inconspicuous type" converted later to "diffuse type."

Immunophenotypic analysis. As shown in Table 3, tumor cells from all 36 cases were determined to be of T-cell nature. E-RFC or CD2 was positive in all cases. CD3 was positive in all 18 cases examined, as was CD5 in all 15 cases examined. In the 23 most recent cases, 12 cases were positive for CD4, six positive for CD8, one negative for both CD4 and CD8, and the phenotype of the remaining four cases could not be determined because of the low percentage of tumor cells. Monoclonal S-Ig was not detected in any of the 22 cases examined. CD20 (B1) was also negative in all of the 14 cases examined. Terminal deoxynucleotidyl transferase (TdT) was detected in none of the seven cases examined. HLA-DR antigen was detected in 21 (84.0%) of 25 cases. Reactions of

### Table 3. Immunophenotypic Analysis of Tumor Cells From Patients With IBL-Like T-Cell Lymphoma

<table>
<thead>
<tr>
<th>E-RFC or CD2</th>
<th>CD5</th>
<th>CD3 (OKT3)</th>
<th>CD4 (OKT4)</th>
<th>CD8 (OKT8)</th>
<th>HLA-DR</th>
<th>CD20 (B1)</th>
<th>S-Ig</th>
<th>TdT</th>
</tr>
</thead>
<tbody>
<tr>
<td>36/36 (100%)</td>
<td>15/15 (100%)</td>
<td>18/18 (100%)</td>
<td>12/23 (52.2%)</td>
<td>6/23 (26.1%)</td>
<td>21/25 (84%)</td>
<td>0/14 (0%)</td>
<td>0/22 (0%)</td>
<td>0/7 (0%)</td>
</tr>
</tbody>
</table>

Abbreviations: E-RFC, rosette-forming capacity with sheep erythrocytes; CD, cluster differentiation; S-Ig, surface immunoglobulin; TdT, terminal deoxynucleotidyl transferase

*CD4 and CD8 monoclonal antibodies were analyzed for the 23 recent cases. Tumor cells in one case lacked CD4 and CD8, while in four other cases the subset expression was indeterminate because of the low percentage of tumor cells.
in five of six "diffuse type" cases, but also in three of four "inconspicuous type" cases.

Treatment and prognosis. The overall survival of the 36 patients with IBL-like T-cell lymphoma is shown in Fig 4. The median survival in these patients was 18 months from diagnosis. The survival of 12 cases of the CD4-positive group was not statistically different from that of six cases of the CD8-positive group.

Detailed information on therapeutic courses was available in 31 of the 36 patients. Table 5 shows the response rates of initial chemotherapy. Chemotherapeutic regimens were divided into three major groups such as steroid hormone alone, cyclophosphamide, vincristine and prednisolone (COP)-containing regimens, and doxorubicin-containing regimens. None of the ten patients treated with a regular dose of prednisolone achieved complete remission. However, one patient receiving high-dose methylprednisolone achieved complete remission. The complete remission rates of COP- and doxorubicin-containing regimens were the same (62.5%). Survival curves of the three groups, such as steroid hormone alone, COP-containing, and doxorubicin-containing regimens were compared, but no significant difference was found (data not shown). Ten of 11 patients who received only steroid hormone as initial therapy had to be treated with combination chemotherapy later. Furthermore, in the eight evaluable patients who received combination chemotherapy as second line therapy after steroid hormone therapy, only two achieved complete remission. One patient showed spon-

Abbreviations: G, germline; R, rearranged; D, deleted.
purified and sequenced as described earlier. Figure 3 at the right illustrates part of the sequencing gel showing that the first nucleotide of codon 61 is an A as well as a T. This suggests that the amplified DNA fraction contained two species, one with an AAG as codon 61 (for lysine) and a second with a TAG as codon 61 (a terminating codon).

To confirm this observation amplified DNA samples from patient 19, his parents (pedigree 4 in Fig 5), and some other individuals were hybridized with synthetic probes; one set of probes was designed to detect the GAG → G-G deletion in codon 6 (this defect was observed on the second chromosome of patient 19; see earlier) and the other to identify the AAG → TAG substitution at codon 61. The data given in Fig 4 nicely illustrate the presence of the codon 6 abnormality in the patient and her mother (and in the Greek control) and that of codon 61 in the patient and her father.

**Family studies.** Family members were included in the evaluation of most patients (excluded are patients 2, 3, 15, and 18), and some pedigrees are shown in Fig 5. The pedigree of patient 10 is of interest because the mother and sister both had S-β-thal (with ~20% Hb A) whereas the father and one brother had simple β-thal traits. Identification of the thalassemia in these subjects was readily made by the dot-blot procedure. Two of the children of patient 17 (second pedigree) inherited the chromosome with the C → T substitution at nt -88, while the third was heterozygous for fl-thal due to the 1.35-kb deletion. The severe microcytosis and high Hb A2 level, earlier noted for a different individual, were again observed. Patient 14 (third pedigree) is also of interest because this child had a relatively low level of Hb F (24.2%) and an unusual haplotype (see the next section). His paternal β-thal relatives (with the A → G mutation at nt -29) had Hb F levels of 2.3%, while his mother (with the T → A mutation at codon 24) had a low Hb F level of 0.5%.

### Table 5. Clinical Responses According to Initial Therapy

<table>
<thead>
<tr>
<th>Cases Evaluable</th>
<th>CR</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>No therapy</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Methylprednisolone (high dose)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>COP-containing Cx</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>DOX-containing Cx</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>28</td>
</tr>
</tbody>
</table>

*Abbreviations: CR, complete remission; PR, partial remission; COP, cyclophosphamide, vincristine, and prednisolone; DOX, doxorubicin; Cx, chemotherapy.

*Spontaneous remission.*
or pale cells of T-cell nature. In addition, both cases showed a progressive clinical course and had to be treated with combination chemotherapy.

Previously, we reported that tumor cells from most IBL-like T-cell lymphoma cases were positive for CD8,\(^4\),\(^8\),\(^15\) However, the present study revealed CD4+ phenotype in 12 of 23 cases, CD8+ phenotype in six, and CD3+, CD4-, CD8- phenotype in one. These results were generally consistent with the immunophenotypic analyses for AILD and "AILD-like lymphoma" by other investigators.\(^10\),\(^33\) Immunophenotypic analyses of IBL-like T-cell lymphoma are sometimes difficult because of the low percentage of tumor cells. However, immune rosette assay\(^17\) and immunohistochemistry\(^18\) were useful for the determination of immunologic phenotype in such cases.

Therefore, IBL-like T-cell lymphoma may be divided into two groups, the CD4-negative group and the CD8-negative group, by surface marker analysis. However, there were no significant differences in clinical manifestations, histologic findings, and survival between both groups (data not shown). In order to clarify the differences between CD4-negative group and CD8-negative group, further studies are necessary.

The prognosis of patients with IBL-like T-cell lymphoma was poor. One of the reasons for the poor prognosis may be a delay in commencement of combination chemotherapy due to diagnostic problems. In the present study, 11 patients received steroid hormone alone as initial therapy with some beneficial effects; however, most of them soon became resistant and had to be treated with combination chemotherapy.

Most of them were initially diagnosed as having AILD or IBL, but after retrospective review, they were diagnosed as IBL-like T-cell lymphoma. A recent investigation on Chinese patients with peripheral T-cell lymphoma revealed that IBL-like T-cell lymphoma occupied almost half of them, and that chemotherapy results of the doxorubicin-containing regimen were significantly better than those of less-intensive regimens.\(^34\) Therefore, there appears to be an improvement in the prognosis of IBL-like T-cell lymphoma with intensive chemotherapy soon after diagnosis. For prompt commencement of intensive chemotherapy, accurate diagnosis is necessary. Histologic analysis, paying attention to pale cells in conjunction with cytologic, immunophenotypic, and immunogenotypic analyses, will help prompt an accurate diagnosis.

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